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Repair of Nitrous Acid Damage to DNA in *Escherichia coli*†

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ABSTRACT: A number of mutant strains of *Escherichia coli* have been examined for their sensitivity to nitrous acid and in some instances to methylmethanesulfonate. All *ung*$^-$ mutants tested are abnormally sensitive to nitrous acid. Since the *ung* mutation is phenotypically expressed as a defect in uracil DNA glycosidase, this observation supports the contention that treatment of cells with nitrous acid causes deamination of cytosine to uracil. In addition the observed sentitivity indicates that the *ung* gene is involved in the repair of uracil in DNA. Studies with other mutants suggest that both exonuclease III and DNA polymerase I of *E. coli* are involved in the repair of nitrous acid damage in vivo.

In recent years a number of laboratories have demonstrated enzyme activities that catalyze the hydrolysis of *N*-glycosidic bonds linking nitrogenous bases to the sugar-phosphate backbone in DNA (Kirtikar and Goldthwait, 1974; Lindahl, 1974, 1976; Friedberg, et al., 1975; Duncan et al., 1976a,b; Katz et al., 1976; Lindahl et al., 1977; Cone et al., 1977). These enzymes (termed DNA *N*-glycosidases (Lindahl et al., 1977) appear to be specific for unusual or modified bases in DNA and may function in initiating DNA repair by releasing the free base from the DNA and creating a site susceptible to attack by apurinic endonucleases.

A uracil DNA glycosidase has been shown in extracts of *E. coli* to attack DNA containing uracil derived by the deamination of cytosine (Lindahl, 1974, 1976; Lindahl et al., 1977). The copolymer (dC, [^{3}H]dU) is also a substrate for this activity (Lindahl, 1974, 1976; Lindahl et al., 1977). A similar (if not identical) activity in extracts of *B. subtilis* has been shown to attack phage PBS2 DNA which naturally contains uracil instead of thymine, as well as the polymer [^{3}H]poly(dU) (Friedberg et al., 1975; Duncan et al., 1976b; Katz et al., 1976). Following infection of *B. subtilis* with phage PBS2, the uracil DNA glycosidase activity disappears due to the induction of a presumably phage-coded inhibitor (Tomita and Takahashi, 1975; Friedberg et al., 1975; Duncan et al., 1976b; Katz et al., 1976). Inhibition of RNA or protein synthesis during phage infection prevents the synthesis of the inhibitor and the phage DNA is rapidly degraded (Duncan and Warner, 1977). When chloramphenicol is added after the inhibitor has been synthesized, the phage DNA remains stable (Duncan and Warner, 1977). These observations suggest that the uracil DNA glycosidase functions in vivo to remove uracil residues from DNA and support a hypothetical biological role for this enzyme in the excision repair of uracil when it occurs in DNA.

It has been reported that treatment of nucleic acids with nitrous acid results in a number of chemical modifications of the DNA, including deamination of cytosine to uracil (Schuster and Schramm, 1958; Schuster, 1960a,b; Litman, 1962; Shapiro and Pohl, 1968; Shapiro and Yamaguchi, 1972). The isolation of mutants of *E. coli* defective in uracil DNA glycosidase (*ung*$^-$) (B. K. Duncan and H. R. Warner, manuscript in preparation) provides an experimental system to test this directly and in the present studies we show that such mutants are abnormally sensitive to nitrous acid treatment. In an effort to gain insights into the molecular mechanisms of subsequent steps in the uracil excision repair pathway(s) in *E. coli*, we have also examined the nitrous acid sensitivity of a number of mutants defective in enzyme activities that might be involved in repair pathways initiated by removal of uracil as the free base (Lindahl, 1976). The sensitivity of a number of these mutants to methylmethanesulfonate has also been tested by ourselves and by Kirtikar et al. (1977).

Material and Methods

1. *Bacterial Strains* (*See Table II*). The wild-type strains AB1157 and W3110 are maintained in our laboratory stocks. The BD series of strains were isolated by us. Strains BD10 and BD13 were independent isolates from a stock of *E. coli* W3110 mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Strain BD1119 is isogenic (by phage P_1 transduction) with an *ung*$^+$ strain BD1117 maintained in the laboratory of H. R. W. A full description of the isolation of the BD strains will be presented elsewhere (B. K. Duncan and H. R. Warner, manuscript in preparation). The BW series of mutants were originally isolated in Dr. B. Weiss's laboratory (Milcarek and Weiss, 1972; Yajko and Weiss, 1975) and were obtained from Dr. David A. Goldthwait, as was strain NH5016, originally isolated by Ljungquist et al. (1976). Strains JG138, JG139, KS463, and RS5069 were obtained from Dr. Priscilla Cooper, Stanford University. All strains were maintained on agar plates containing 1% tryptone (Difco), 0.5% yeast extract (Difco), 1% sodium chloride, and 1.5% agar. Strains KS463 and

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RS5069 were maintained and grown on plates containing medium without sodium chloride since the presence of salt enhances their lethality (Cooper, 1977).

2. *Measurement of Sensitivity to Nitrous Acid.* Bacteria were grown to log phase (100 Klett units) from overnight stationary phase cultures in double strength nutrient broth (Difco) at 37 °C. Cells were harvested by centrifugation at 7000*g* for 5 min and resuspended in 4.0 mL of double strength nutrient broth at a concentration of 10^9 cells/mL. Nitrous acid was prepared as follows: 2.0 M sodium nitrite was prepared in double distilled water; 6.0 mL of this solution (prepared fresh for every experiment) was mixed with 10 mL of 1.0 M acetic acid, 5 mL of 1.0 M sodium acetate, and 39.0 mL of distilled water. This mixture constitutes a solution of 0.2 M nitrous acid; 10.0 mM nitrous acid was prepared at room temperature by adding 1.0 mL of 0.2 M nitrous acid to 19.0 mL of 0.2 M sodium acetate buffer (pH 4.3). This solution was filter sterilized through a Nalgene filter unit (Nalgene Sybron Corp., Rochester, N.Y.). One milliliter of cells (at 10^9 cells/mL) was mixed with 9.0 mL of sterile 10 mM nitrous acid giving a final concentration of 10^8 cells/mL in 9.0 mM nitrous acid. Incubations were at room temperature for varying periods of time. Control samples were incubated in 9.0 mM sodium acetate buffer (pH 4.3) under identical conditions. Treatment was terminated by diluting 1.0 mL of each cell suspension into 9.0 mL of 0.1 M Tris[1]-HCl buffer (pH 7.5). The bacteria were serially diluted in 0.1 M Tris-HCl (pH 7.5) and plated on yeast extract–tryptone agar. Colonies were scored after incubation at either 25, 37, or 42 °C in different experiments.

3. *Treatment with Methylmethanesulfonate.* Cultures in log phase at a concentration of 5×10^8 cells/mL were prepared as described above and incubated at room temperature in the presence of methylmethanesulfonate (Sigma Chemical Co.) (2 μg/mL). Treatment was terminated by a 10^{-3}-dilution into 0.1 M Tris-HCl buffer at pH 7.5. Dilutions were plated and colonies scored as described above.

4. *Treatment with Mitomycin C.* Cultures in log phase at a concentration of 5×10^8 cells/mL were prepared as described above and incubated at room temperature in the presence of mitomycin C (Sigma Chemical Co.) (2 μg/mL). Treatment was terminated by a 10^{-3} dilution into 0.1 M Tris-HCl buffer at pH 7.5. Dilutions were plated and colonies scored as described above.

5. *Ultraviolet Irradiation.* Cells were resuspended in 0.1 M Tris-HCl buffer (pH 7.5) at a concentration of 10^8/mL. Aliquots (5.0 mL) were irradiated with a General Electric low-pressure mercury vapor lamp in 60 × 0.5 mm Petri dishes with constant stirring. The incident fluence rate was 2.5 J m^{-2} s^{-1} as determined with an IL 254 germicidal photometer (International Light). Irradiated cultures were plated and scored as described above.

6. *Enzyme Assays.* Cells were grown to stationary phase in double strength nutrient broth and harvested by centrifugation at 7000*g* for 15 min. Cells were rinsed in 10 mM Tris-HCl buffer (pH 7.5), resuspended in this buffer, and sonicated with a Biosonic IV sonicator using a 3/8-in. diameter probe. The extract was centrifuged at 15 000*g* for 15 min and the supernatant used as a crude enzyme fraction. Uracil DNA glycosidase activity was assayed by the procedure of Duncan et al. (1976b). Incubation mixtures (0.15 mL) contained [^{3}H]poly(dU) (75 pmol as nucleotide), 10 mM Tris-HCl buffer (pH 7.5), 25 mM NaCl, 1 mM EDTA, and 0.2–20 μg of protein. Incubations were for 20 min at 37 °C. DNA polymerase activity was measured by the procedure of Sedwick et al. (1972). Incubation mixtures (0.25 mL) contained activated salmon sperm DNA (150 nmol as nucleotide) as a template–primer, the four usual deoxynucleoside triphosphates (each at 0.1 mM), 20 mM $MgCl_2$, 2 mM β-mercaptoethanol, 0.2 mg of bovine serum albumin, 10 mM Tris-HCl buffer (pH 8.9), [^{3}H]TTP (4 μCi/mL; specific activity 0.04 μCi/nmol), and 0.1–0.4 mg of protein. Incubations were at 37 °C for 10 min.

7. *Protein Determinations.* Protein concentration in crude extracts was measured by the procedure of Lowry et al. (1951).

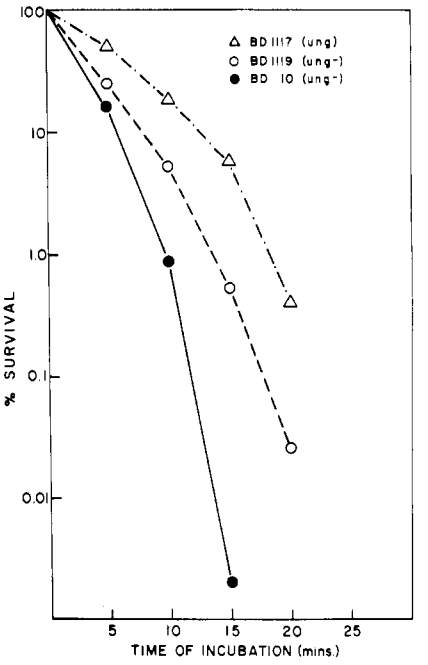

FIGURE 1: Sensitivity of wild-type and *ung*⁻ mutants to treatment with nitrous acid. Strains BD1117 and BD1119 are an isogenic pair. Further experimental details are provided in the text.

Results

The survival of four independently isolated uracil DNA glycosidase mutants was compared with wild-type cells following treatment with nitrous acid prepared and used as described above, or with sodium acetate at pH 4.3. Figure 1 shows a representative experiment. Nitrous acid is toxic to wild-type bacteria, since only 10% of the cells survive treatment for 15 min under the conditions used here. Control incubations in acetate buffer at pH 4.3 resulted in less than 10% killing (data not shown). All strains carrying the *ung*⁻ allele are always more sensitive to nitrous acid than wild-type strains; however, it was consistently observed that both strains BD10 and BD13 (primary isolates with other mutations in the genetic background) were the most sensitive *ung*⁻ strains. To minimize the possible contribution of other mutations, the BD10 *ung*⁻ allele was transduced into a wild-type genetic background to produce essentially isogenic strains. These strains too were more sensitive to nitrous acid treatment than the wild-type parents (Figure 1). We conclude that the increased nitrous acid sensitivity of strain BD1119 is due to the *ung* mutation. The results obtained with strains BD10 and BD13 suggest that these strains are additionally defective in some other gene(s) required for the repair of nitrous acid damage to DNA. None of the

[1] Abbreviations used: Tris, tris(hydroxymethyl)aminomethane; UV, ultraviolet; EDTA, ethylenediaminetetraacetic acid; MMS, methylmethanesulfonate.

TABLE I: Percent Survival Values Following Treatment with MMS, UV, or Mitomycin C.[a]

Strain	MMS Incubation time (min) 10	15	20	30	UV Irradiation time (min) 10	20	40	50	Mitomycin C Incubation time (min) 10	20	30
W 3110 (*ung*$^+$)	60	27	12	2							
BD 1117 (*ung*$^+$)	46	30	6	0.8							
BD 1126 (*ung*$^+$)					23	4.6	0.66	0.12	10	5.5	1.9
BD 13 (*ung*$^-$)	47	26	14	0.9							
BD 10 (*ung*$^-$)	33	15	12	1.3	23	3	1	0.08	15	4.4	2.5
BD 1119 (*ung*$^-$)	52	13	4.5	1.2							
BD 1121 (*ung*$^-$)					26	8	0.8	0.11	8	4.6	1.1

[a] Experimental details are described fully in the section on Materials and Methods.

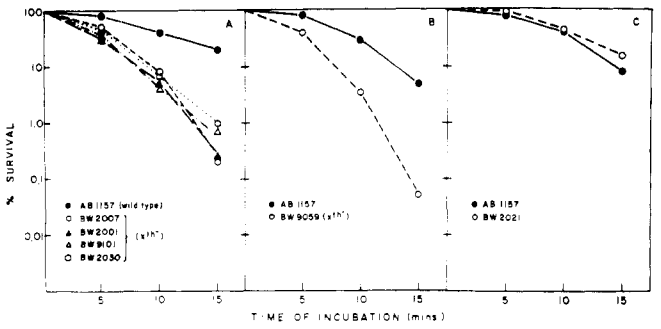

FIGURE 2: Sensitivity of wild-type and x^{th} gene mutants to treatment with nitrous acid. Experimental details are provided in the text.

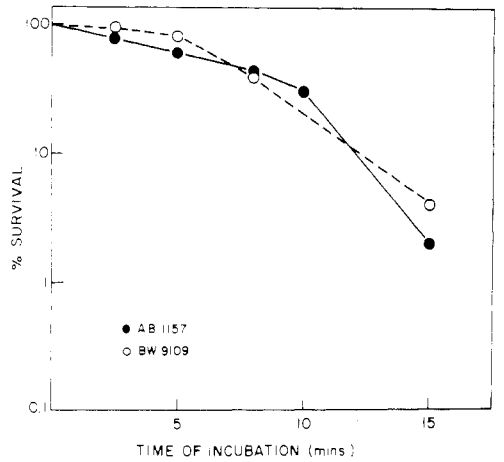

FIGURE 3: Comparative survival of strains AB1157 (wild-type) and BW 9109 (x^{th-}) to nitrous acid treatment.

ung$^-$ mutants tested showed an abnormal sensitivity to either methylmethanesulfonate treatment, UV radiation, mitomycin C (Table I), or acetate buffer at pH 4.5.

Lindahl (1976) has proposed a pathway for the excision repair of uracil in DNA involving the action of the uracil DNA glycosidase, followed by endonucleolytic attack at the site of base loss, exonucleolytic degradation of the sugar-phosphage residue, repair synthesis to restore missing nucleotides and DNA rejoining. In view of the results described above, we decided to examine the nitrous acid sensitivity of mutants reported to be defective in some of these biochemical reactions (De Lucia and Cairns, 1969; Konrad and Lehman, 1975; Milcarek and Weiss, 1972; Yajko and Weiss, 1975; Kirtikar et al., 1976a,b; Ljungquist et al., 1976).

A number of mutants isolated in Weiss's laboratory termed x^{th-} mutants have been reported to be defective in exonuclease III (Milcarek and Weiss, 1972, 1973; Yajko and Weiss, 1975;

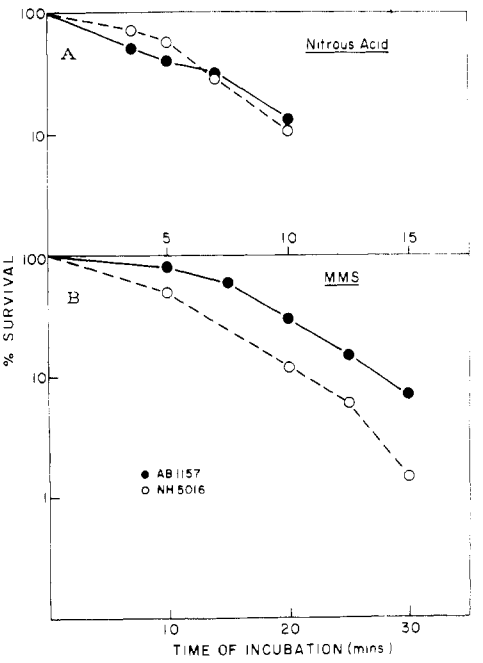

FIGURE 4: Comparative sensitivity of stains AB1157 (wild-type) and NH5016 to nitrous acid and methylmethanesulfonate.

White et al., 1976). However, the precise phenotype of these mutants remains controversial. Weiss (1976) has suggested that exonuclease III and endonuclease II (defined by its activity on heavily alkylated DNA) are the same enzyme. Studies from Goldthwait's laboratory are in contradiction, indicating that endonuclease II, exonuclease III, and apurinic endonuclease are distinct activities that can be chromatographically separated (Kirtikar et al., 1976a,b, 1977). As shown in Figures 2A and 2B, five different x^{th-} mutants tested are abnormally sensitive to nitrous acid. Strain BW2021, a methylmethanesulfonate resistant revertant of BW2001 and reported by both Yajko and Weiss (1975) and Kirtikar et al. (1977) to have normal enzyme levels, showed a survival curve like that of the wild-type control strain (Figure 2C).

Strain BW9109 is a derivative of strain BW9101, obtained by transduction into a wild-type genetic background. The latter strain carries a deletion that includes the region $x^{th} \rightarrow pnc$ A (Yakjo and Weiss, 1975). As shown in Figure 2A, strain BW9101 is abnormally sensitive to nitrous acid. Surprisingly, however, strain BW9109 is not abnormally sensitive to nitrous acid in our hands (Figure 3). Another x^{th} gene mutant that displays a normal level of sensitivity to nitrous acid is strain NH5016 (Figure 4A). This mutant was derived by transduction of strain AB3027, a strain highly sensitive to methylmethanesulfonate. As shown by Ljungquist et al. (1976), strain

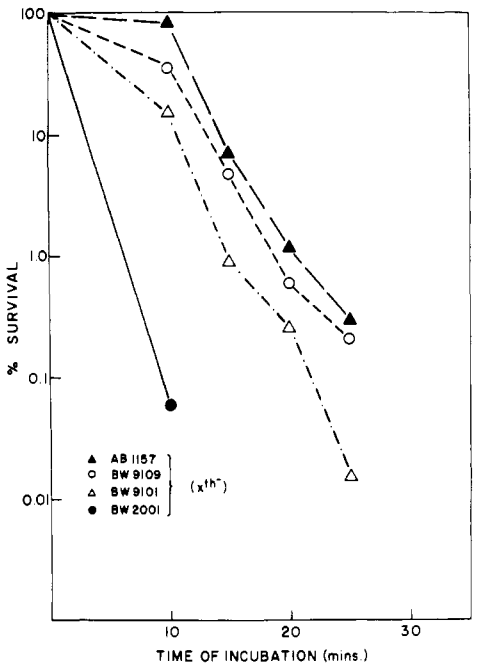

FIGURE 5: Sensitivity of wild-type and x^{th} gene mutants to treatment with methylmethansulfonate. Experimental details are provided in the text.

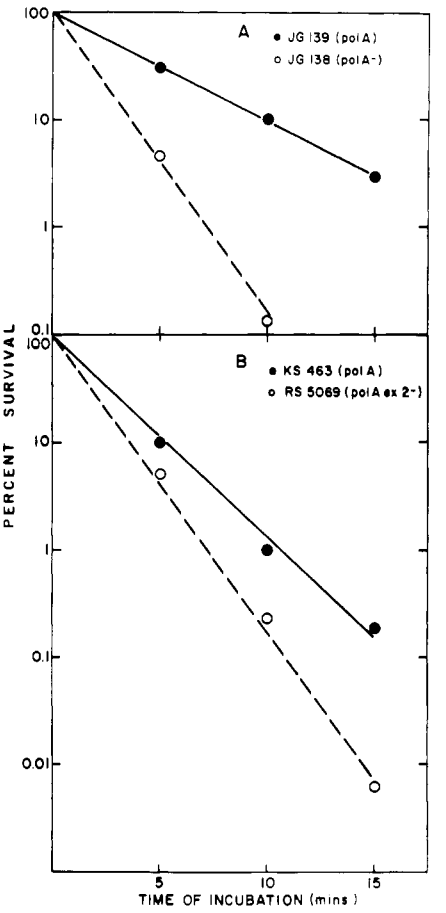

FIGURE 6: Sensitivity of DNA polymerase I defective mutants to nitrous acid. Experimental details are provided in the text.

AB3027 carries the *pol* A mutation, the loss of which in the tranduced derivative NH5016 results in a significant but not complete reduction in MMS sensitivity compared with the parent strain.

The x^{th} mutation of AB3027 has been mapped at the x^{th} A locus (White et al., 1976) and shown to be exonuclease III defective (Yajko and Weiss, 1975). Strain NH5016 has been shown to be defective in a major apurinic endonuclease activity associated with exonuclease III (Ljungquist et al., 1976; Lindahl, 1977) and to be defective in exonuclease III by Kirtikar et al. (1977). We therefore assume that strain NH5016 still carries the x^{th} A mutation but have not directly measured exonuclease III activity ourselves.

With respect to methylmethanesulfonate sensitivity of the x^{th} mutants, in our hands strain BW 2001 is markedly sensitive (Figure 5), in agreement with the data of Yajko and Weiss (1975) and of Kirtikar et al. (1977). However, strain BW9101, reported by Kirtikar et al. (1977) to be no more sensitive to methylmethanesulfonate than the wild-type strain AB1157, is distinctly more sensitive in our hands, though less sensitive than BW2001 (Figure 5). Both x^{th} mutants that are insensitive to nitrous acid (strains BW9109 and NH5016) are very slightly sensitive to methylmethanesulfonate (Figures 5 and 4B, respectively).

Finally, we have observed that the *pol* A mutant JG138 is considerably more sensitive to nitrous acid than its isogenic parent JG139 (Figure 6A). Strain RS5069 (*pol* A *ex* 2) [defective in the 5′→3′-exonuclease activity of DNA polymerase I (Konrad and Lehman, 1974)] is also abnormally sensitive to nitrous acid compared with its isogenic wild-type parent KS463. However, the differential survival between mutant and wild-type strains is smaller than with the *pol* A strains (Figure 6B). The significantly increased killing of strain KS463 compared with JG138 may be a consequence of the modified plating conditions used to prevent expression of the salt sensitivity of strain RS5069 (Cooper, 1977). All of the x^{th} gene mutants used in this study as well as strain NH5016 were found to have normal levels of DNA polymerase I and uracil DNA glycosidase in vitro (Table II). Strains BD10, BD13, BD1119, and BD1121 (all ung^-) were also shown to contain normal levels of DNA polymerase but had less than 1% the wild-type level of uracil DNA glycosidase (Table II).

Discussion

Since the observation that thymine-containing cyclobutyl-pyrimidine dimers are removed from the acid-insoluble fraction of DNA in UV-irradiated bacteria (Boyce and Howard-Flanders, 1964; Setlow and Carrier, 1964; Riklis, 1965), a general model of excision repair of damaged or inappropriate bases has been postulated. This model, extensively discussed in the literature (Setlow, 1968; Howard-Flanders, 1968; Strauss, 1968; Hanawalt, 1968; Grossman, 1974; Grossman et al., 1975), postulates that excision repair occurs by the action of an endonuclease that recognizes abnormalities in DNA, followed by exonucleolytic degradation resulting in the excision of the damaged base(s) as nucleotides. Experimental support for such a model of excision repair is quite extensive. Both endonuclease and exonuclease activities capable of carrying out these functions in vitro have been isolated and characterized from a number of sources (Grossman, 1974; Grossman et al., 1975; Friedberg, 1975). In addition, mutants defective in some of these activities have been shown to be abnormally sensitive to certain forms of DNA damage (Setlow, 1968; Howard-Flanders, 1968; Strauss, 1968; Hanawalt, 1968; Grossman, 1974; Grossman, et al., 1975; Friedberg, 1975; Cooper, 1977). Finally, evidence has been provided that indicates that pyrimidine dimers are excised as nucleotides; generally part of a larger oligonucleotide (Pawl et al., 1975). It has

TABLE II: Relevant Genotype and Phenotype of Bacterial Strains.

Strain	Relevant genotype	Rel spec act. of Uracil DNA glycosidase	Rel spec act. of DNA polymerase[a]
W 3110	W.T.	100	100
BD 10	*ung*⁻	<1.0	
BD 13	*ung*⁻	<1.0	
BD 1117	*ung*⁺ } isogenic	116	
BD 119	*ung*⁻ } isogenic	<1.0	
BD 1126	*ung*⁺ } isogenic	108	
BD 1121	*ung*⁻ } isogenic	<1.0	
AB 1157	W.T.	100	100
BW 2007	x^{th-}	100	136
BW 2030	x^{th-}	86	111
BW 2001	x^{th-}	116	71
BW 2021	x^{th+} revertant		
BW 9101	Spontaneous deletion x^{th} → *pnc* A	134	100
BW 9109	P_1 tranductant x^{th} → *pnc* A		
BW 9059	x^{th-}	99	108
BW 9094	x^{th-}		
BW 9097	x^{th-}		
NH 5016	?		
JG 139	*pol* A⁺ } isogenic		
JG 138	*pol* A⁻ } isogenic		
KS 463	*pol* A⁺ } isogenic		
RS 5069	*pol* A⁻ *ex*2⁻ } isogenic		

[a] Only x^{th} gene mutants sensitive to nitrous acid were assayed for uracil DNA *N*-glycosidase and DNA polymerase. The *ung* mutants were not assayed for DNA polymerase activity since none of them were sensitive to methylmethanesulfonate. The specific activities are normalized to the % values obtained in the wild-type strains.

been proposed that this mode of excision repair be designated as nucleotide excision repair (Duncan et al., 1976b; Friedberg et al., 1977).

More recently evidence has accumulated suggesting the existence of an alternative mode of base excision involving the release of the free base by the action of DNA glycosidase activity. The first report of such an activity was by Kirtikar and Goldthwait (1974) who showed that an enzyme designated as endonuclease II of *E. coli* (Friedberg and Goldthwait, 1969) catalyzed the release of O^6-methylguanine and 3-methyladenine from DNA reacted with *N*-methyl-*N*-nitrosourea. Subsequently it was shown that this enzyme also promoted the release of N^6-(12-methylbenz[*a*]anthracenyl-7-methyl)adenine and N^2-(12-methylbenz[*a*]anthracenyl-7-methylbenz[*a*]anthracenyl-7-methyl)guanine (Kirtikar et al., 1975). Recently, Lindahl (1977) has described the purification and characterization of a DNA glycosidase from *E. coli* that catalyzes the release of 3-methyladenine but not O^6-methylguanine from alkylated DNA. The relationship of this enzyme to endonuclease II is not clear. A second base excision repair enzyme was first reported by Lindahl (1974) in *E. coli* and by Friedberg et al. (1975) in *B. subtilis* and is termed uracil DNA glycosidase (Lindahl et al., 1977) since it catalyzes the release of free uracil from deoxyribonucleic acid or deoxyribopolymers bearing uracil bases.

Although uracil does not usually exist naturally in DNA (phages PBS1 and PBS2 being interesting exceptions), this base could theoretically arise in DNA as the result of incorporation from dUTP by DNA polymerase, or as the result of the deamination of cytosine in DNA. With respect to the former mechanism, the pool size of dUTP is presumably an important determinant. Recent studies by Tye et al. (1977) suggest that mutants defective in dUTP hydrolase activity may incorporate uracil into their DNA, although this has not been directly shown. As regards cytosine deamination, Shapiro and Klein (1966) found that free cytosine and cytidine are relatively rapidly deaminated by heating in weakly acidic buffers. Lindahl and Nyberg (1974) showed that cytosine in single-strained DNA, poly(dC) or dCMP is similarly susceptible to heat-induced deamination, the reaction proceeding at a rate constant of 2×10^{-7} s^{-1} at 95 °C and pH 7.4. In native DNA the cytosine residues were better protected and deamination occured at less than 1% of the rate observed with dCMP or poly(dC). In addition to heat, treatment of cytidine, cytidine 5′-phosphate, poly(cytidylic acid), and poly(I)·poly(C) with nitrous acid (Shapiro and Yamaguchi, 1972) and of deoxycytidine, deoxycytidylic acid or single-stranded DNA with sodium bisulfite also promoted deamination to uracil (Shapiro et al., 1973).

In the present studies we have shown that mutants defective in the enzyme uracil DNA glycosidase are abnormally sensitive to treatment with nitrous acid. This sensitivity appears to be specific, since treatment with sodium acetate at pH 4.5, UV irradiation, methylmethanesulfonate or mitomycin C does not result in preferential killing compared with wild-type strains. These results are consistent with the notion that the DNA glycosidase is involved in the excision repair of uracil in DNA, possibly catalyzing the first step in the repair process. Nitrous acid damage to DNA including deaminated cytosine may also be repaired by a nucleotide excision repair mode. In this regard it is of interest to note that Gates and Linn (1977) have described an enzyme in *E. coli* designated endonuclease V, that extensively degrades PBS2 DNA to oligonucleotides. Free uracil is not a product of this reaction, however.

Following removal of free uracil from DNA, how is the repair process completed? One theoretical possibility is that the apyrimidinic site is repaired by direct reinsertion of the appropriate base. To our knowledge no evidence for such a mechanism exists and we have been unable to detect an activity that transfers thymine to deuracilated PBS2 DNA from either thymidine, TMP, TDP, or TPP (Simmons and Friedberg, unpublished observations). A more realistic possibility based on existing enzymologic data is that an apyrimidinic site is attacked by an endonuclease that recognizes sites of base loss in DNA. Consequent exonucleolytic degradation could excise the deoxyribose-phosphate residue allowing for repair synthesis and DNA rejoining. Based on this model, the observed sensitivity of the *pol* A mutant is not unexpected and argues for a role of this enzyme in the resynthesis step. The results obtained with the *pol* A *ex* 2 mutant which is defective mainly in the 5′→3′-exonuclease activity of DNA polymerase I (Konrad and Lehman, 1974) suggest that this enzyme may also play a role in exonucleolytic degradation in the 5′→3′ direction during repair. On the other hand, the *pol* A *ex* 2 mutant shows a slightly decreased activity of DNA polymerase I dependent polymerizing activity (I. R. Lehman, personal communication) which might explain the smaller sensitivity of this strain to nitrous acid (relative to the wild-type parent) as compared with that of the *pol* A_1 mutant.

All of the x^{th} gene mutants that we studied that are abnormally sensitive to nitrous acid are defective in exonuclease III activity. This result could reflect a requirement for 3′-exonuclease activity, 3′-phosphatase activity, or apurinic endonuclease activity during the repair of nitrous acid damage to DNA. The former two are activities originally associated with exonuclease III (Richardson et al., 1964). More recently, Yajko and Weiss (1975), Weiss (1976) and Ljungquist and Lindahl (1977) have reported evidence that exonuclease III has an associated apurinic endonuclease activity. The latter

authors suggest that this represents the major apurinic endonuclease activity in *E. coli*.

On the other hand, Kirtikar et al., (1976,a,b; 1977) have measured exonuclease III activity (defined by its 3′ phosphatase and exonuclease function), an apurinic endonuclease activity (acting on depurinated DNA) and endonuclease II activity (acting on alkylated DNA) and have reported the chromatographic resolution of these activities in both wild-type and mutant strains. This disagreement suggests that the phenotype of the x^{th} gene mutants may not be fully resolved and at the present time complicates an understanding of the role of exonuclease III in DNA repair.

In our hands not all strains defective in exonuclease III activity are abnormally sensitive to nitrous acid, notable exceptions being strains BW9109 and NH5016. The former strain is a deletion mutant in which the extent of the deletion may critically differ from that in the parent strain BW9101, which *is* abnormally sensitive to nitrous acid. If so, it is apparent that the genetic regulation of the repair of nitrous acid damage is quite complex. Until this complexity is resolved, we can offer no obvious explanation for the results obtained with strains BW9109 and NH5016.

In conclusion we have evidence that uracil DNA glycosidase is involved in the repair of nitrous acid damage to DNA (presumably the deamination of cytosine to uracil). Studies in progress are aimed at examining the kinetics of uracil excision from the DNA of ung^+ and ung^- cells treated with nitrous acid. A complete understanding of the molecular mechanism of the later stages of uracil excision repair must await a clearer understanding of the enzymologic defects in the x^{th} gene mutants and the isolation of further mutants defective in single enzyme activities.

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