Characterization and Genetic Mapping of a Mutation Affecting Apurinic Endonuclease Activity in *Staphylococcus aureus*

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Received 10 March 1986/Accepted 11 August 1986

Protoplast fusion between the Rec⁻ mutant RN981 (L. Wyman, R. V. Goering, and R. P. Novick, Genetics 76:681-702, 1974) of *Staphylococcus aureus* NCTC 8325 and a Rec⁺ NCTC 8325 derivative yielded Rec⁺ recombinants that exhibited the increased sensitivity to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine characteristic of RN981. Transformation analyses identified a specific mutation, designated *ngr-374*, that was responsible not only for *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine sensitivity, but also sensitivity to methyl methanesulfonate, ethyl methanesulfonate, nitrous acid, and UV irradiation. However, *ngr-374*-carrying recombinants showed no significant increase in their sensitivity to mitomycin C or 4-nitroquinoline 1-oxide and were unaffected in recombination proficiency. In vitro assays showed that *ngr-374*-carrying strains had lower apurinic/apyrimidinic endonuclease activities than the wild type. The chromosomal locus occupied by *ngr-374* was shown to exist in the gene order $\Omega(\text{Chr::Tn551})40\text{-}n\text{gr-374}\text{-thrB106}$.

Apurinic and apyrimidinic (AP) sites in DNA arise either from spontaneous depurination and depyrimidation or by the activity of DNA-*N*-glycosylases (19). AP sites can be either mutagenic or lethal to a cell (34), but efficient repair minimizes the deleterious effects of these sites (33, 43). Repair of AP sites occurs by base excision repair, which in many aspects resembles nucleotide excision repair of UV-induced DNA dimers (8, 19, 21, 46). Base excision repair of AP sites requires endonucleases that are specific for AP sites (19, 41). Many such endonucleases from both procaryotes and eucaryotes have been isolated; they are collectively referred to as AP endonucleases. The most extensively studied AP endonucleases are those of *Escherichia coli* (for a review, see reference 20).

In *E. coli*, three endonucleases with AP endonuclease activity have been characterized. Exonuclease III is responsible for 90% of the AP endonucleolytic activity in *E. coli*, and endonucleases III and IV account for the remainder (13, 22, 44). Generally, exonuclease III-deficient mutants, which carry the *xthA* mutation, are sensitive to methyl methanesulfonate (MMS) and nitrous acid, but are unchanged in their sensitivity to UV irradiation (4, 8, 23). Although these mutants lack the major AP endonuclease, they show no increase in their spontaneous mutation rate (49). Apparently, the minor AP endonucleases, endonucleases III and IV, supply sufficient AP endonucleolytic activity to repair the spontaneously occurring AP sites; however, they are unable to manage the increased number of AP sites formed with MMS or nitrous acid treatments (22, 44, 46).

In this report, we describe *ngr-374*, a mutation impairing AP endonuclease activity and originating in the recombination-deficient (Rec⁻) mutant RN981 (48) of *Staphylococcus aureus* NCTC 8325. *ngr-374*, isolated during experiments to characterize the genetic basis of Rec⁻ in RN981, has been mapped by transformation. Strains carrying *ngr-374* are similar to *E. coli* mutants carrying *xthA*, which causes increased sensitivity to DNA-alkylating agents and nitrous

acid. *ngr-374*-carrying strains were also sensitive to UV irradiation, suggesting that *S. aureus* may have an AP endonuclease-dependent DNA repair mechanism for UV-generated DNA lesions.

MATERIALS AND METHODS

Bacterial strains. The strains of *S. aureus*, *Bacillus subtilis*, and *E. coli* used in this study are described in Table 1. All cultures were maintained as described previously (35).

Culture media. All commercially available dehydrated culture media were supplemented with thymine (20 µg/ml) and adenine, guanine, cytosine, and uracil (5 µg/ml each). The composition of complete defined synthetic agar (31) was modified by omitting the appropriate amino acids, purines, and pyrimidines as needed to score and select nutritional markers (2, 29, 30). *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) agar was prepared by adding 5 µg of MNNG (Sigma Chemical Co.), freshly dissolved in absolute methanol, per ml to autoclaved, cooled (45°C) Trypticase soy agar (TSA; BBL Microbiology Systems) rehydrated in 100 mM KH₂PO₄, pH 6.0 (10). MNNG agar was used on the day of preparation. MMS agar was TSA to which 5 µg of MMS (Sigma) per ml was added immediately before pouring into petri dishes. Antibiotic resistance phenotypes were selected and scored on brain heart infusion agar (BHI; Difco Laboratories) containing the appropriate concentration of antibiotic (2, 36). Methicillin resistance (Mec^r) was scored on TSA plus 5% (wt/vol) NaCl and 6.25 µg of methicillin per ml (26).

Protoplast fusions and transformation of *S. aureus*. Protoplast fusions, transformations with high-molecular-weight transforming DNA, and analysis of recombinants were performed as described by Stahl and Pattee (35). Sensitivity or resistance to MNNG (the *Ngr* phenotype) was scored on MNNG agar.

Inactivation assays. Cultures were grown overnight at 37°C on BHI agar slants, and the resulting cells were added to 10 ml of the appropriate assay medium to a final concentration of 10⁷ CFU/ml. These cells were then incubated at 37°C. Samples were removed at intervals and assayed for CFU on BHI agar at 37°C after incubation for 24 h.

MNNG inactivation assays were performed in 0.85% sodium citrate, pH 5.0. MMS inactivation assays were

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TABLE 1. Bacterial strains used

Strain	Genotype or phenotype	Origin or reference
<i>S. aureus</i>		
ISP8	8325-4 <i>pig-131</i>	39
ISP39	8325 <i>thy-101 purA102 pig-131</i>	31
ISP387	8325 <i>trp-103</i> Ω (Chr::Tn551) Δ 40 <i>ilv-129 pig-131</i>	ISP374 (30) DNA $\times$ ISP14 ^a
ISP926	8325 <i>r1⁻ r2⁻ m31⁺ M32⁺ lys-115 trp-103 thrB106 ala-126 tmn-3106 ilv-129 pig-131 hisG15 nov-142</i>	35
ISP1061	8325-4 <i>pig-131 recA1 his-7</i> (pI258 <i>blaI401 mer-14 repA36</i>)	ϕ 80 α /ISP479 (29) $\times$ RN1030 (48) ^b
ISP1171	8325 <i>thrB106 uraA141 ilv-129 mec-4916 nov-142 pig-131 ala-126 trpE85 tyrB282 ermB321 tmn-3106 ϕ11^s ϕ12^s</i>	ISP92 (28) DNA $\times$ ISP1169 ^c
ISP1185	8325-4 <i>pig-131 tyrA362::Tn551 recA1 his-7</i>	ISP1061 $\times$ 43°C ^d
ISP1238	8325 <i>r1⁻ r2⁻ m31⁺ m32⁺ lys-115 trp-103 ala-126 tmn-3106 ilv-129 pig-131 hisG15 nov-142 ngr-374</i>	ISP1185 DNA $\times$ ISP926
ISP1297	8325-4 <i>pig-131 thrB106</i>	ISP5 (31) DNA $\times$ ISP835 (32)
ISP1308	8325-4 <i>pig-131 ngr-374</i> Ω (Chr::Tn551) Δ 40	ISP1295 ^e DNA $\times$ ISP1297
ISP1358	8325 <i>r1⁻ r2⁻ m31⁺ m32⁺ lys-115 thrB106 ala-126 tmn-3106 ilv-129 pig-131 hisG15 nov-142 ngr-374</i>	ϕ 80 α /ISP2 $\times$ ISP1344 ^f
RN981	8325-4 <i>recA1 his-7 pig-131</i>	48 ^g
RN1441	8325-4 <i>his-7 mit-6 pig-131</i>	48 ^h
<i>B. subtilis</i>		
W23	Wild type	50
SB68	168 <i>trpC2 hisH2 tyrA1</i>	BGSC ⁱ
<i>E. coli</i> K-12		
IDMS5	Wild type	IDM ^j
BW9109	F ⁻ <i>thr-1 ara-14 leuB6</i> Δ (<i>gpt-proA</i>)62 <i>lacY1 tsx-33 supE44 galK2 λ^- Rac⁻ Δ(<i>xthA-pncA</i>)90 hisG4 rfbD1 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1</i>	CGSC ^k

^a For example, ISP14 was transformed with DNA isolated from ISP374. ISP14 = 8325 *trp-103 thrB106 ilv-129 pig-131*.

^b For example, RN1030 was transduced with phage 80 α propagated on ISP479.

^c ISP1169 = 8325 *thrB106 uraA141 ilv-129 mec-4916 nov-142 pig-131 ala-126 trpE85 tyrB282 ermB321* Ω (Chr::Tn551) Δ 7 ϕ 11^s ϕ 12^s.

^d Isolated from ISP1061 by transposon Tn551 mutagenesis as described by Pattee (29).

^e ISP1295 = 8325 *r1⁻ r2⁻ m31⁺ M32⁺ lys-115 trp-103 ala-126 tmn-3106 ilv-129 pig-131 hisG15 nov-142 ngr-374* Ω (Chr::Tn551) Δ 40.

^f ISP1344 = 8325 *r1⁻ r2⁻ m31⁺ m32⁺ lys-115 trp-263::Tn551 thrB106 ala-126 tmn-3106 ilv-129 pig-131 hisG15 nov-142 ngr-374*.

^g Received from R. V. Goering, Creighton University School of Medicine, Omaha, Neb.

^h Received from R. P. Novick, Public Health Research Institute of the City of New York, Inc., New York, N.Y.

ⁱ Received from the Bacillus Genetic Stock Center (BGSC), Ohio State University, Columbus, Ohio (catalog no. 1A87).

^j Iowa State University teaching collection.

^k Received from the *E. coli* Genetic Stock Center (CGSC), Yale University, Hartford, Conn. (CGSC strain 6063).

performed in Trypticase soy broth, pH 7.3 (TSB; BBL Microbiology Systems). Nitrous acid inactivation assays were performed in a modification of the medium described by Karren et al. (17), consisting of 4.75 ml of TSB and 0.25 ml of freshly prepared 0.2 M NaNO₂ in 0.1 M sodium acetate, pH 4.6. UV sensitivity was determined in saline (0.85% NaCl) with a 15-W germicidal lamp (Sylvania Electric Products Inc.).

Disk assays. Disk assays were adapted for *S. aureus* from the assay described by Ljungquist et al. (23). Cultures were grown overnight on BHI agar slants; an estimated 2×10^8 cells were added to 4.0 ml of melted, cooled (45°C) soft TSA (TSB plus 0.4% agar), which was poured onto TSA supplemented with 5 mM CaCl₂. After 4 h at room temperature, a 1.25-cm-diameter sterile filter paper disk (Schleicher & Schuell) was placed on the surface of each plate, and 20 μ l of the agent being assayed was applied to the disk. After overnight incubation at 37°C, the diameters of the zones of inhibition were measured.

***S. aureus* cell extracts.** Cell extracts were isolated from 100 ml of mid- to late-log-phase cells grown in TSB at 37°C. The cells were harvested, washed once in saline, suspended in 10

ml of SMTB (35) containing 3 mg of lysostaphin (Sigma) per ml, and incubated at 37°C for 30 min. The resulting protoplasts were pelleted at $3,500 \times g$ for 5 min and suspended in 0.02 to 0.05 ml of Hadden buffer (10% sucrose in 50 mM Tris hydrochloride [Sigma], 15 mM dithiothreitol [Sigma], 200 mM NaCl, and 1 mM phenylmethylsulfonyl fluoride, pH 8.0 [14]). The protoplast suspension was placed on ice, and Brij 58 (Sigma) was added to a final concentration of 0.2%, followed by incubation at 37°C for 3 min. The resulting lysate was centrifuged at $300,000 \times g$ (SW60 rotor; Beckman Instruments, Inc., Palo Alto, Calif.) for 60 min at 4°C, and the supernatant (cell extract) was stored at -70°C. After adding 0.2 ml of extract to 0.05 ml of 1.5 N NaOH and boiling for 10 min (R. E. Hurlebert, personal communication), protein was assayed by the method of Lowry et al. (25).

Bioassay of AP endonuclease activity. The bioassay of AP endonuclease activity in cell extracts was done as described by Ljungquist et al. (23). DNA was isolated from overnight cultures of *B. subtilis* W23 grown in 100 ml of Penassay broth no. 3 (Difco) as described by Lovett and Keggins (24). The DNA was dissolved in 0.15 M NaCl-0.015 M sodium citrate,

pH 5.0, adjusted to 19 µg of DNA per ml, and depurinated by heating at 70°C for 110 min. Competent cells of the indicator strain for these bioassays, *B. subtilis* SB68, were prepared by the protocol of Dubnau and Davidoff-Abelson (6). Competent cells were stored at -70°C in SpII medium containing 10% (vol/vol) glycerol (6).

The bioassay conditions were as described by Ljungquist et al. (23). Cell extracts were adjusted to the desired protein concentrations with Hadden buffer, and 5 µl was added to 100 µl of HEPES buffer (100 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid]-KOH [Nutritional Biochemicals Corp.] and 3 mM MgCl₂, pH 8.5). The bioassay was started by adding 20 µl of depurinated DNA and incubation at 37°C. After 15 min, the bioassay was stopped by adding 5 µl of 0.2 M EDTA, pH 7.0, and heating the bioassay mixture at 70°C for 3 min. Each cooled bioassay mixture received 0.5 ml of competent SB68 cells and then was incubated for 30 min at 37°C. SB68 Trp⁺ transformants were assayed on tryptophan-deficient complete defined synthetic agar (31), which was incubated at 37°C for 48 h.

In vitro AP endonuclease activity assay. AP endonuclease activity was also assayed by measuring the release of acid-soluble material from depurinated DNA as described by Verly and Rassart (41, 42). DNA from protoplasts of strain ISP39 previously grown in complete defined synthetic broth (31) containing [³H]thymidine was extracted, alkylated, and depurinated with MMS, followed by heating as described by Verly (41), and stored at -20°C in 0.05 M Tris hydrochloride-0.15 M NaCl-0.015 M trisodium citrate, pH 7.0. Samples (40 µl; ca. 26,000 dpm/ml) of depurinated DNA were added to 100 µl of cell extract in 0.5 M Tris hydrochloride-0.1 M EDTA-0.1 mM β-mercaptoethanol, pH 8.0, and incubated for 15 min, after which the DNA was precipitated with 900 µl of 10% perchloric acid, and the acid-soluble counts were assayed by liquid scintillation.

RESULTS

Genetic mapping of the *ngr* mutation. The original objective of this study was to define the chromosomal location of the genetic determinant(s) of the Rec⁻ phenotype in *S. aureus* 8325. Because the Ngr^s phenotype can be used to isolate Rec⁻ mutants (10), the Ngr^s phenotype and

FIG. 1. Linkage of Ω(Chr::Tn551)40, *ngr*-374, and *thrB106* in *S. aureus* as determined by transformations. Map distances were calculated from the averages of the data shown in Table 2 and a transformation analysis in which ISP1297 was transformed with ISP1295 DNA. Map distances (in map units) are presented as estimated cotransformation frequencies of unselected markers. Arrows point from selected to unselected markers.

protoplast fusion (35) were used in attempts to determine the approximate chromosomal location of the Rec⁻ determinant. Protoplast fusions between strains ISP1185 and ISP1171 (Table 1) showed that the Ngr phenotype preferentially segregated with the Thr phenotype, suggesting that the Ngr^s determinant was located near *thrB*. The chromosomal location of the Ngr^s determinant (designated *ngr*-374) was established in a series of transformation experiments that were possible only when the modification-deficient nature of RN981 (48) was taken into account by using recipient strains derived from the restrictionless strain 8325 mutant SA113 (r1⁻ m31⁺ r2⁻ m32⁺ [16]). One transformation experiment is summarized in Table 2, and the map location of *ngr*-374 is shown in Fig. 1.

Because *ngr*-374 originated in strain RN981, strain RN1441, the spontaneous Rec⁺ Ngr^s revertant of RN981 (48), was examined for the presence of *ngr*-374. RN1441 was transformed with DNA from ISP387, which carries Ω(Chr::Tn551)40, with selection for Em^r. Among the Em^r transformants recovered, 21% were also Ngr^r (data not shown). These results show that *ngr*-374 in RN981 was linked by transformation to Ω(Chr::Tn551)40 and that *ngr*-374 does not impair recombination, a conclusion also

TABLE 2. Analysis of the *thrB106*, *ngr*-374, and Ω(Chr::Tn551)40 determinants by transformation of ISP1358 (Em^s Thr⁻ Ngr^s) with DNA from ISP387 (Em^r Thr⁺ Ngr^r)^a; distribution of recombinants observed

Class	Phenotype ^b	% of recombinants ^c with selection for:	
		Em ^r	Thr ⁺
A	Em ^r Ngr ^r Thr ⁻	10	— ^d
B	Em ^r Ngr ^s Thr ⁺	1	2.5
C	Em ^s Ngr ^s Thr ⁺	—	33
D	Em ^s Ngr ^r Thr ⁺	—	6.5
E	Em ^s Ngr ^s Thr ⁻	28	—
F	Em ^s Ngr ^r Thr ⁺	61	58

^a The transformation frequency was 3,415 Em^r transformants and 995 Thr⁺ transformants per 10⁹ CFU, and the reversion frequency was <10 revertants per 10⁹ CFU for both selections; 10⁹ CFU and excess DNA were used per ml of transformation suspension.

^b See Table 1 for genotypes. Em^r, Erythromycin resistance conferred by Ω(Chr::Tn551)40; Ngr^r, resistance to MNNG; Thr⁺, L-threonine independence.

^c 683 Em^r and 415 Thr⁺ recombinants were analyzed for unselected markers.

^d —, Not observed.

FIG. 2. Inactivation assays of ISP926 (*ngr*⁺) (■) and ISP1238 (*ngr*-374) (▲); response to (A) MNNG (100 µg/ml) and (B) MMS (5 mM).

TABLE 3. Disk assay of *S. aureus* sensitivity to various DNA-modifying agents

Strain	Ngr phenotype	Prophage content	Zone of inhibition (mm) ^a			
			EMS	MMS	MitC	NQO
ISP8	Ngr ^r	None	0	20	20	0
ISP926	Ngr ^r	φ11, φ12, φ13	0	54	40	25
ISP1185	Ngr ^s	φ11	39	62	35	30
ISP1238	Ngr ^s	φ11, φ12, φ13	23	62	40	26
ISP1297	Ngr ^r	None	0	20	26	0
ISP1308	Ngr ^s	None	23	54	28	0

^a Values are the diameters of the zones of inhibition from a single experiment.

borne out by many other transformations in which Ngr^s recipient strains were used.

Phenotypes of *ngr-374*. The degree of sensitivity to MNNG imposed by *ngr-374* was determined by inactivation assays with ISP1238 and ISP926. Strain ISP1238 (Ngr^s) was more sensitive to MNNG (100 μg/ml) than ISP926 (Ngr^r; Fig. 2A). To determine whether this sensitivity was limited to MNNG, these inactivation assays were repeated with MMS, which produces a totally different spectrum of DNA products (37). ISP1238 was also more sensitive than ISP926 to MMS (Fig. 2B). These data suggested that *ngr-374* impaired the repair of alkylated DNA. Based on the *E. coli* model for alkylated DNA repair, *ngr-374* was thought to be responsible for a deficiency in either a DNA-glycosylase such as 3-methyl-N-glycosylase, an AP endonuclease, or the enzymes for gap repair (21).

To determine the spectrum of *ngr-374*-induced sensitivity, various DNA-modifying agents were examined by disk assays. The results (Table 3) indicate that *ngr-374*-carrying strains were sensitive only to the DNA alkylating agents ethyl methanesulfonate (EMS) and MMS, and relatively resistant to mitomycin C (MitC) and 4-nitroquinoline 1-oxide (NQO). Even ISP1238, which is lysogenic and experienced prophage induction by MMS, MitC, and NQO, was visibly more sensitive to the DNA-alkylating agents (Table 3) than its wild-type parent, ISP926. These results support the

FIG. 3. Inactivation assays of ISP926 (*ngr*⁺) (■) and ISP1238 (*ngr-374*) (▲); response to 50 mM nitrous acid.

notion that *ngr-374* affected a repair pathway specific for alkylated DNA.

Host cell reactivation (HCR) experiments were used to test for a deficiency in a DNA-N-glycosylase specific for products common to MNNG, MMS, and EMS. Bacteriophage 80α treated with 50 mM MMS and then assayed for PFU on ISP926 and ISP1238 was reactivated to the same extent by both strains (data not shown). If *ngr-374* was a mutation in a DNA-N-glycosylase specific to a product common to MMS, EMS, and MNNG treatments, then the ability of *ngr-374*-carrying strains to reactivate MMS-treated bacteriophage should have been curtailed (17, 38). However, the similarity in the reactivation activities of these strains strongly suggested that *ngr-374* did not affect such a DNA-N-glycosylase.

Since it appeared that *ngr-374* did not affect a DNA-N-glycosylase specific for alkylated DNA, the *ngr-374*-carrying strains were examined for loss of AP endonuclease activity. Da Roza et al. (4) demonstrated that *E. coli* mutants deficient in the major AP endonuclease (*xthA*) were sensitive to nitrous acid treatment. Treatment of DNA with nitrous acid deaminates cytosine and adenine (17), and these deaminated bases are removed enzymatically, resulting in the formation of AP sites. In *E. coli*, it was assumed that *xthA*-imposed sensitivity to nitrous acid primarily reflected an inability to repair these AP sites (4). ISP1238 showed a consistent twofold-higher sensitivity to nitrous acid treatment than the wild-type strain ISP926. Furthermore, the ISP1238 survival rate decreased in a linear fashion, while the rate for ISP926 decreased curvilinearly. The shape of these curves suggested that in ISP1238 a single lesion produced by nitrous acid was lethal, while repair of these lesions occurred in ISP926. Linear regression analysis gave correlation coeffi-

FIG. 4. Bioassay of AP endonuclease activity in ISP926 (*ngr*⁺) and ISP1238 (*ngr-374*). AP endonuclease activity was assayed by treating depurinated W23 DNA with different concentrations of cell extract from ISP926 (▲) and ISP1238 (●) and determining the effect of these treatments on the transformation activity of the DNA. Transformation activity is expressed as percent Trp⁺ transformants recovered, with 100% equal to the number of Trp⁺ transformants recovered from transformations with depurinated W23 DNA not treated with cell extract. Between 10⁵ and 10⁶ Trp⁺ transformants per ml were recovered from the untreated depurinated DNA. The effect on the transformation activity of control (nondepurinated) W23 DNA treated with ISP926 (△) and ISP1238 (○) cell extracts is also shown.

TABLE 4. AP endonuclease activity in cell extracts from *S. aureus* and *E. coli* assayed against ³H-labeled apurinic DNA

Amount of protein in assay (μg)	% of acid-soluble cpm released ^a with extracts from:			
	<i>S. aureus</i> ^b		<i>E. coli</i> ^c	
	ISP926 ^d (<i>ngr</i> ⁺)	ISP1238 ^e (<i>ngr</i> -374)	K-12 (<i>xth</i> ⁺)	BW9109 (<i>xth</i> deletion)
0	0	0	0	0
50	8.0	3.0	19.1	0.8
100	19.9	10.4	19.9	0.3
200	— ^f	—	51.0	3.8
500	22.1	13.0	—	—
1,000	31.1	21.5	—	—

^a Percent released minus percent released when no protein was added to the assay mixture.

^b Values are the averages of 3, 3, 2, 1, and 1 experiment with 0, 50, 100, 500, and 1,000 μg of protein, respectively.

^c Values are for a single experiment at each protein concentration.

^d With labeled DNA that was not depurinated, between 0.2 and 0.9% of the label was converted to acid-soluble form.

^e With labeled DNA that was not depurinated, between 0.0 and 0.4% of the label was converted to acid-soluble form.

^f —, Not determined.

cients of -0.95 and -0.80 for ISP1238 and ISP926, respectively, which were supportive of the plots as they are shown (Fig. 3). Based on these data, it appeared that *ngr*-374 caused a deficiency in AP endonuclease activity.

Assays for AP endonuclease activity in cell extracts. The AP endonuclease activity in cell extracts of ISP926 and ISP1238 was determined by the *B. subtilis* bioassay described by Ljungquist et al. (23). In this assay, DNA from *B. subtilis* W23 was depurinated in vitro and used to transform strain SB68. When depurinated DNA is treated with AP endonuclease, nicks are introduced into the DNA at the depurinated (AP) sites. These nicks cause a reduction in the transformation activity of the depurinated DNA, and the severity of the reduction indicates the AP endonuclease activity in the cell extract being assayed.

Depurinated DNA from strain W23 treated with cell extracts from ISP926 exhibited a rapid drop in transforming activity at low protein concentrations (Fig. 4). In contrast, depurinated DNA treated with cell extracts from ISP1238 showed only a slight reduction in transformation activity at protein concentrations below 50 μg/ml, indicating that the *ngr*-374-carrying strain had a lower level of AP endonuclease activity than the wild type. However, when the amount of ISP1238 cell extract was increased to 50 μg or more of protein per ml, the transforming activity dropped precipitously. This rapid loss in transforming activity was attributed to the recovery of AP endonuclease activity due to either leakiness in the *ngr*-374 mutation or complementation by other nucleases present in the extracts.

The AP endonuclease activity in cell extracts was also assayed by measuring the release of acid-soluble material from depurinated DNA. Samples of ³H-labeled depurinated DNA from ISP39 were incubated with cell extracts of *Ngr*^s and *Ngr*^r *S. aureus* strains, after which the acid-soluble counts were assayed. The assays were repeated with nondepurinated DNA and with cell extracts prepared from *E. coli* K-12 strains known to differ in AP endonuclease activity (47). The results (Table 4) are consistent with those of the transformation assay: at low concentrations of cell extract from the *ngr*-374-carrying strain, reduced endonucleolytic activity was evident compared with that in the wild type. At higher concentrations of protein, the difference between the two *S. aureus* strains lessened. In

FIG. 5. UV inactivation of ISP1297 (○) and ISP1308 (●). Strains were irradiated with 0.6 J/m² per s from a 15-W germicidal lamp.

contrast, the *E. coli* cell extracts exhibited a sharp contrast in activity, consistent with previous reports (42).

UV sensitivity of *ngr*-374-carrying strains. The AP endonuclease mutants of *E. coli* (*xthA*) are unchanged in their sensitivity to UV irradiation (4, 8, 23). When the nonlysogenic *ngr*-374-carrying strain ISP1308 and the *Ngr*⁺ parent strain ISP1297 (Table 1) were examined for their UV sensitivity, ISP1308 was about twice as sensitive to UV irradiation as the wild type (Fig. 5). These results demonstrate that the *ngr*-374 mutation causes sensitivity not only to DNA-alkylating agents, but also to UV irradiation. The significance of this UV sensitivity with regard to the other data presented is discussed below.

DISCUSSION

The results of this study demonstrate the efficacy of protoplast fusion in circumventing the *Rec*⁻ phenotype of RN981 and in identifying one mutational lesion (*ngr*-374) that contributes to the phenotype of this strain. Once the approximate chromosomal location of *ngr*-374 was identified by protoplast fusion, transformation was used to move this mutation to a clean genetic background, where it was further characterized genetically and biochemically.

Recombinants that acquired *ngr*-374 by transformation showed increased sensitivity to DNA-alkylating agents, nitrous acid, and UV irradiation, but remained unchanged in their sensitivity to MitC and NQO. Neither genetic recombination nor cell viability appeared to be impaired by *ngr*-374. Based on current models of DNA repair, DNA lesions generated by alkylating agents and UV irradiation are repaired by base excision and nucleotide excision, respectively (7, 19, 46). The sensitivity of *ngr*-374-carrying strains to alkylating agents and nitrous acid suggests that they are deficient in AP endonuclease activity (4, 7, 8, 17), and both assays for AP endonuclease showed that *ngr*-374 lowered the activity of this enzyme. However, these assays also demonstrated AP endonuclease activity in *ngr*-374-carrying strains when increasing amounts of cell extract were used.

This apparent recovery of AP endonuclease activity resembles that reported by Ljungquist et al. (23) for *xthA* mutations in *E. coli*. They concluded that the exonuclease III deficiency caused by *xthA* was compensated for by minor AP endonucleases in the cell extract (23, 45, 46). The results of HCR experiments with MMS-treated bacteriophage 80 α , showing that the *ngr-374*-carrying strains were unaffected in HCR activity, were similar to the HCR results reported for *E. coli xthA* mutations, which were used as further evidence for compensation of the exonuclease III deficiency by minor AP endonucleases (17, 27). This compensation of the exonuclease III deficiency has been used to explain why cell viability was unaffected in *xthA*-carrying strains under normal growth conditions (23). Based on our data, it appears that *ngr-374* is similar to *xthA* in that *ngr-374* causes a reduction in AP endonuclease activity. Under normal growth conditions this deficiency seems to be compensated for by other nucleases in *S. aureus*.

While most of our data indicate that *ngr-374* is a mutation affecting AP endonuclease activity, the UV sensitivity of the *ngr-374*-carrying strains creates a paradox. The repair of UV-generated DNA lesions is usually thought to occur by nucleotide excision repair (7, 19), a process which does not generate AP sites and does not require an AP endonuclease (7). However, there are several cases in which AP endonucleolytic activity has been detected in the repair of UV-generated DNA lesions, including the UV endonucleases of coliphage T4 (27) and *Micrococcus luteus* (12, 40) and more recently the repair of UV-generated thymine glycol (1, 5, 20). Based on these reports, it is possible that *S. aureus* may also have an AP endonuclease-dependent repair mechanism for UV-generated lesions.

Evidence for this view is based mainly on the response of *ngr-374*-carrying strains to NQO. NQO mimics the biological effect of UV irradiation so closely that mutants impaired in UV excision repair in *E. coli* and *B. subtilis* are sensitive to this compound (3, 9, 15, 18). Similarly, *uvr* mutants of *S. aureus*, like their *E. coli* and *B. subtilis* counterparts, are impaired in UV excision repair (except *uvr-1* and *uvr-8* mutants [11]) and are also sensitive to NQO (unpublished results). In contrast, *ngr-374*-carrying strains were not sensitive to NQO, indicating that *ngr-374* was not related to the *uvr* mutations. Goering and Pattee (11) also showed that the *uvr* mutants were unable to reactivate UV-irradiated bacteriophage. However, Wyman et al. (48) reported that neither RN981 nor the Rec⁺ revertant of this mutant, RN1441, was impaired in its ability to reactivate UV-irradiated bacteriophage; both strains carry *ngr-374*. The *ngr-374*-carrying strains failed to demonstrate any of the phenotypes characteristic of *uvr* mutations; therefore, it would appear that the UV sensitivity caused by *ngr-374* may be due to an AP endonuclease-dependent UV repair process in *S. aureus* which is independent of the UV excision repair pathway affected by the *uvr* mutations described by Goering and Pattee (11).

In summary, we have identified and mapped the mutation *ngr-374*, which, judging from the phenotypes, affects AP endonuclease activity in *S. aureus*. It is unknown whether *ngr-374* is a mutation in the gene encoding the AP endonuclease or in a gene which affects its enzymatic activity. While *ngr-374* behaves very similarly to the *xthA* mutations in *E. coli*, *ngr-374*, unlike *xthA* mutations, causes sensitivity to UV irradiation. This UV sensitivity implies that *S. aureus* has an AP endonuclease-dependent UV repair process which is distinct from the UV excision repair process detected by Goering and Pattee (11).

ACKNOWLEDGMENTS

This investigation was supported by National Science Foundation grants PCM-8110058 and PCM-8310681 and by Public Health Service grant AI-06647 from the National Institute of Allergy and Infectious Diseases.

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