Adaptive Response and Enhancement of N-Methyl-N'-Nitro-N-Nitrosoguanidine Mutagenesis by Chloramphenicol in Streptomyces fradiae

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Streptomyces fradiae expressed an adaptive response to treatment with small doses of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) that caused a reduction in mutagenesis by treatment with larger doses of MNNG. Treatment of S. fradiae with high levels of MNNG in the presence of chloramphenicol caused enhancement of mutagenesis, independent of the adaptive response.

N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) is a potent mutagenic agent for many microorganisms (4, 6), including Streptomyces species (2, 3a, 5, 7, 14, 15, 18). Because MNNG is the most potent mutagen of those studied in Streptomyces species (3a, 18), it is a very useful agent for inducing mutations to improve antibiotic yields. There are at least two ways that MNNG can be used to induce mutations in Streptomyces species. Cells can be treated with a high concentration of mutagen (100 to 600 µg/ml) for a short period (20 to 30 min) followed by segregation in the absence of mutagen (2, 18); alternatively, cells can be treated with a low concentration of mutagen for an extended period followed by segregation. A possible drawback to the former approach is that the treatment time may be substantially less than the cell doubling time, which is about 90 to 150 min for fast-growing Streptomyces species (1). Because MNNG preferentially mutates at the replication fork in Escherichia coli (4) and probably in Streptomyces species (7, 14, 15), a short treatment might result in preferential mutagenesis in a limited portion of the genomes in individual cells. However, because most mutagenesis in Streptomyces fradiae by MNNG requires error-prone DNA repair (18), high levels of MNNG may be required to fully induce the error-prone repair enzyme(s). Treatment of Streptomyces species with a low concentration of MNNG for a lengthy period might induce an adaptive response, similar to that expressed in E. coli and Bacillus subtilis. The adaptive response in these bacteria renders the induced cells less mutable and more resistant to the lethal effects of MNNG during subsequent treatment with larger doses (10-13, 19). The adaptive response to mutagenesis in these microorganisms is due primarily to the induction of a methyltransferase enzyme that removes methyl groups from O6-methylguanine and O4- methylthymine; the enzyme thus reverses the potentially mutagenic reaction of MNNG with guanine and thymine residues in DNA (10, 11). The adaptive response in E. coli also includes a DNA glycosylase which removes O2-methylcytosine, O2-methylthymine, and N6-methylated purines (11). If streptomycetes encode similar enzymes, treatment with small doses of MNNG for extended periods might result in reduced levels of mutagenesis.

To determine whether S. fradiae, a producer of the macrolide antibiotic tylosin (3), expresses an adaptive response, cells were treated with 0.5 and 1.0 µg of MNNG per ml for 4 h and then challenged with larger doses of MNNG. In the absence of adaptation, treatment of S. fradiae with 5 to 20 µg of MNNG per ml for 20 min caused the induction of mutations for resistance to spectinomycin (Spc'; Fig. 1A). When cells were pretreated with 0.5 or 1.0 µg of MNNG per ml, no mutation induction was observed after subsequent treatments with 5 to 20 µg of MNNG per ml for 20 min (Fig. 1A). These small doses of MNNG do not cause any reduction in cell viability (18). Therefore, S. fradiae encodes an inducible adaptive response that presumably repairs potentially mutagenic genetic lesions caused by MNNG.

To determine whether higher levels of MNNG pretreatment of cells could also induce an adaptive response, S. fradiae M1 was treated with 5 µg of MNNG per ml for 4 h and then was challenged with a dose of 50 µg of MNNG per ml for 0 to 30 min. The adapting dose did not result in reduced mutagenesis in the subsequent challenge period, but it caused a high level of mutagenesis during the adaptation period (Fig. 1B). The challenge with 50 µg of MNNG per ml caused an additional threefold increase in mutagenesis to Spc' at a frequency of 7.5 × 10⁻³; this was at least threefold higher than the maximum frequency for induced mutations obtained in the controls that did not receive the adapting treatment. It appears that treatment of S. fradiae M1 with 5 µg of MNNG per ml for 4 h caused the accumulation of too many potentially mutagenic lesions for an adaptive response system to repair and probably caused the induction of an error-prone repair pathway required for most MNNG mutagenesis (3a, 18).

In E. coli, treatment of cells with chloramphenicol during MNNG treatment causes an increase in mutagenesis and reduces the frequency of multiple mutations in the region of the replication fork (16). This increased mutagenesis has been attributed to an inhibition of the adaptive response (17). We treated S. fradiae with MNNG in the presence of chloramphenicol and observed a 10-fold increase in mutation to Spc' and rifamycin resistance (Rif'; Fig. 2). If chloramphenicol prevented the induction of an adaptive response in S. fradiae, it should have had little or no effect on frequencies of mutation at high concentrations of MNNG, because the adaptive response was effective only at very low concentrations of MNNG (Fig. 1). If chloramphenicol treatment prevented the induction of an error-prone DNA repair system, which accounts for about 90% of MNNG-induced mutagenesis in S. fradiae (18), then it should have caused a reduction in induced mutation. Also, if chloramphenicol...

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inhibited the induction of a major error-free DNA repair system, then a reduction in viability might have been expected, but this was not observed (Fig. 2). Our results suggest that another mechanism must account for the enhanced mutagenesis in the presence of chloramphenicol. In E. coli the recA protein, which is involved in the induction of the umuC protein required for error-prone repair (19), is also actively involved in the error-prone repair process (20). In the presence or absence of chloramphenicol, the recA protein can be converted to a modified form (recA* that is involved in error-prone mutagenesis and stable DNA replication (reinitiation of DNA synthesis in the presence of chloramphenicol [8-10]). Stable DNA replication with a recA* protein bound to the replication complex or interacting with replication in some other way might be more error prone than normal replication. Perhaps in S. fradiae chloramphenicol inhibits reinitiation of normal DNA synthesis but allows reinitiation of a more error-prone stable DNA replication.

![Graph](http://jb.asm.org/)

**FIG. 1.** Adaptive response of S. fradiae M1 to low levels of MNNG. (A) S. fradiae M1 (I) was grown to stationary phase (about 1,400 Klett U) and sonicated to obtain single CFUs (I). The sonicated mycelia were diluted in Trypticase soy broth (I) to approximately 350 Klett U. A 50-ml sample of the cell suspension was added to three 250-ml Erlenmeyer flasks. MNNG was added to the flasks at 0.5, and 1.0 µg/ml, and the cultures were incubated for 4 h at 37°C in a New Brunswick water bath shaker at about 270 rpm. The cells were washed by centrifugation and resuspended in 50 ml of Trypticase soy broth, incubated with MNNG at 0, 5, 10, and 20 µg/ml for 20 min, washed again, and incubated for 24 h at 37°C to allow for segregation (2). The frequency of Spc' mutants was determined as previously described (18). Symbols: ○, unadapted cells; Δ, cells adapted with 0.5 µg of MNNG per ml for 4 h; □, cells adapted with 1.0 µg of MNNG per ml for 4 h. (B) S. fradiae M1 was grown and treated with MNNG as described in panel A, except that MNNG was added at 5 µg/ml for adaptation. The adapted or unadapted cells were then treated with 50 µg of MNNG per ml for 0 to 30 min, and the frequency of Spc' mutants was determined. Symbols: ○, unadapted cells; Δ, cells adapted with 5 µg of MNNG per ml for 4 h.

![Graph](http://jb.asm.org/)

**FIG. 2.** Induction of Spc' and Rif' mutants by MNNG in the presence of chloramphenicol. S. fradiae M1 was treated with MNNG for 20 min as previously described (2, 18), except that chloramphenicol was added at 50 µg/ml during the treatment period. Symbols: △, frequency of Spc' mutants without chloramphenicol; ◻, frequency of Spc' mutants with chloramphenicol; ○, frequency of Rif' mutants without chloramphenicol; ●, frequency of Rif' mutants with chloramphenicol; ■, surviving fraction of viable cells without chloramphenicol.

Our results show that S. fradiae has an adaptive response to very low levels of MNNG but that this repair system is probably not relevant for practical applications of mutagenesis for antibiotic yield improvement. The adaptive response in S. fradiae is apparently saturated at intermediate-sized doses of MNNG and dominated by an error-prone repair system (18) that facilitates efficient mutagenesis. The further augmentation of MNNG mutagenesis by treatment in the presence of chloramphenicol may be very useful for inducing specific rare mutations when the resulting strains can be backcrossed to eliminate extraneous mutations.

We thank Cheryl Alexander for typing the manuscript.

**LITERATURE CITED**


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