Radiomimetic Properties of 2-Aminopurine in *Escherichia coli*

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ABSTRACT

ZAMPIERI, ANTONIO (Palo Alto Medical Research Foundation, Palo Alto, Calif.), AND JOSEPH GREENBERG. Radiomimetic properties of 2-aminopurine in *Escherichia coli*. J. Bacteriol. **91**:1773-1774. 1966.—A radioresistant mutant (R_4) of *Escherichia coli* strain S was found to be resistant to 2-aminopurine. The composition of the post-treatment plating medium and temperature of incubation did not influence the sensitivity of R_4 to 2-aminopurine. However, strain S was much more sensitive to 2-aminopurine when plated on tryptone-agar than on minimal glucose-salts-agar. Strain S was also more sensitive when the post-treatment incubation was at 37 C than at 45 C. When *E. coli* strain S was treated with sufficient 2-aminopurine to permit only 10^{-5} survivors, 67% of the survivors were resistant to ultraviolet light and to radiomimetic compounds. 2-Aminopurine induced filaments in radiation-sensitive, but not radiation-resistant, strains of *E. coli*. It is concluded that 2-aminopurine, a base analogue which induces transition mutations, is radiomimetic in several properties.

Mutants of *Escherichia coli* S isolated from among survivors of treatment with any radiomimetic agent display increased resistance to the selecting agent, and cross-resistance to ultraviolet (UV) radiation and to other (radiomimetic) chemicals. The damage inflicted on the bacterial deoxyribonucleic acid (DNA) by various radiomimetic chemicals, which inactivate bacterial cells by different mechanisms, is reversible by post-treatment conditions that also permit partial recovery of UV-inactivated cells (6). Moreover, the radiomimetic agents share other biological properties with UV light, such as causing filament formation in sensitive strains, induction of lysogenic bacteria, inactivation of transforming DNA, and mutagenesis (3, 5). If a chemical has one of these biological properties, it can usually be shown to possess most, if not all, of the others. On this basis we have examined 2-aminopurine, a base analogue mutagen known to induce transition mutations (2), for other radiomimetic properties. We will show that mutants of *E. coli* selected for resistance to 2-aminopurine are cross-resistant with UV, that

2-aminopurine causes filament formation, and that damage from it is subject to modification by post-treatment conditions such as the composition of the plating medium and temperature of post-treatment incubation.

MATERIALS AND METHODS

The strains of bacteria used and their source, derivation, and properties, as well as the chemicals and media used and most of the methods, have been described in detail (4). 2-Aminopurine was purchased from Cyclo Chemical Corp., Los Angeles, Calif. To test for sensitivity to 2-aminopurine, bacteria were grown overnight in peptone (Difco) broth, washed twice in buffered saline, and diluted to 10^7 bacteria per milliliter of tryptone (Difco) broth (pH 7.0) containing 15 mg/ml of 2-aminopurine. A 1-ml amount of the preparation in a 50-ml flask was incubated in a shaking water bath at 37 C and sampled at intervals. The samples were diluted in buffered saline, plated on appropriate medium, and incubated at 37 C for 18 to 24 hr. The terminal, 60-min sample was plated in quadruplicate on tryptone-agar; two of the plates were incubated at 37 C and two at 45 C.

RESULTS

The inactivation of strains S and R_4 by 2-aminopurine (15 mg/ml) was exponential when the strains were plated on tryptone-agar. The

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slope of the survival curve of strain R_4 was 0.35 of the slope of strain S plated on tryptone-agar. *E. coli* S was more resistant to 2-aminopurine when plated on M9 than when plated on tryptone-agar. The reduction in slope resulting from plating on M9 was from 0.85 to 0.50. On the other hand, the sensitivity of R_4 was the same on both of the two plating media. The radioresistant mutant R_3 displayed the same degree of resistance to 2-aminopurine as did R_4 . Moreover, like R_4 , the rate of survival of R_3 bacterial cells was not affected by plating medium.

When the sample, withdrawn after 60 min of treatment with 2-aminopurine, was plated in quadruplicate on tryptone-agar, the number of surviving cells of strain S was about 10 times greater after incubation at 45 C (3.8×10^{-3}) than at 37 C (4×10^{-4}). On the other hand, R_4 displayed the same degree of sensitivity at either 37 or 45 C (6.0×10^{-2} and 5.7×10^{-2} surviving cells, respectively). (Results are an average of four to five experiments.) Strain R_3 was unaffected by temperature of post-treatment incubation.

E. coli S was plated on tryptone-agar containing 1.0 mg/ml of 2-aminopurine, and was incubated overnight at 37 C. Of 176 colonies (10^{-6} survivors) tested by the rapid method for detecting resistance to ultraviolet light (4), 118 (67%) were found to be resistant to UV. The UV-resistant mutants were tested by the gradient plate method for their resistance to 1-methyl-3-nitro-1-nitrosoguanidine, mitomycin C, and nitrogen mustard; 62 (52%) were identical in their cross-resistance pattern with the radioresistant type R_4 , and 56 (48%) with the type R_3 (4).

Bacteria grown overnight in peptone broth were washed twice in buffered saline and diluted to 10^7 bacteria per milliliter in peptone broth containing 15 mg/ml of 2-aminopurine. A 1-ml amount of the preparation was incubated stationary at 37 C overnight. At the end of the treatment, a smear of each culture was made and stained with crystal violet, and the morphology of the cells was examined microscopically. Filaments were observed after the treatment of strain S with 2-aminopurine, but, as demonstrated previously with other radiomimetic agents (1), there was no induction of filaments after the exposure of the mutant R_4 to 2-aminopurine.

DISCUSSION

We have shown that a mutant of *E. coli* S resistant to UV and cross-resistant with such radiomimetic chemicals as nitrogen mustard and mitomycin C is also resistant to 2-aminopurine.

Furthermore, mutants were isolated at high frequency from survivors of treatment with 2-aminopurine which were resistant to UV, nitrosoguanidine, mitomycin, and nitrogen mustard. All of these resistant mutants resembled in their cross-resistance pattern one of two previously isolated radioresistant mutants of strain S. As with UV and with radiomimetic chemicals, a portion of the lethal damage inflicted by 2-aminopurine on strain S but not its radioresistant mutants was reversible either by plating on minimal medium or by incubating at an elevated temperature. Finally, *E. coli* S, but not its radioresistant mutant, became filamentous as a result of exposure to 2-aminopurine, which resembles UV in this respect. Thus, 2-aminopurine, a potent mutagen known to induce mutations by causing transitions, i.e., the substitution of one purine or pyrimidine by another, can be considered a radiomimetic agent. Some of the mechanisms responsible for resistance to UV and to such chemicals as nitrogen mustard, mitomycin C, and 1-methyl-3-nitro-1-nitrosoguanidine must also be responsible for resistance to 2-aminopurine.

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