Effect of Genotype on Mutagenicity of Niridazole in Nitroreductase-deficient Bacteria

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ABSTRACT

The mutagenicity of niridazole for Salmonella typhimurium depends upon the enzymic reduction of the nitro function. The response of niridazole nitroreductase-deficient bacteria to niridazole is reduced to 4.4 and 0.19% that exhibited by the enzyme-proficient parent strain when the deficiency is the result of a base substitution and frame-shift mutation, respectively. The results are taken to indicate that the residual activity (4.4%) seen in the strain with a base substitution mutation reflects the activity of an enzyme with an amino acid substitution, while the basal level (0.19%) of activity indicates the action of a different nitroreductase with a low specificity for niridazole.

INTRODUCTION

Earlier work in our laboratory demonstrated the value of nitroreductase-deficient bacteria in determining the biochemical basis for the mutagenicity of nitroaromatic drugs and environmental agents (4, 19–23). Subsequent studies have led to the realization that Salmonella tester microorganisms possess a family of nitroreductases with differing substrate specificities (19, 20). This observation suggested the necessity of reevaluating many of the earlier experimental results obtained at a time when it was postulated that these tester microorganisms possessed only one type of nitroreductase with a broad specificity.

We have recently investigated the chemical basis of the mutagenicity of niridazole (4), a therapeutically useful anthelminthic agent (7). That study utilized a niridazole-resistant nitroreductase-deficient Salmonella tester strain derived from Salmonella tester strains TA98 and TA100. We demonstrated that the parent nitroreductase-proficient Salmonella tester strain was very sensitive to the mutagenic and antimicrobial activity of niridazole, while the nitroreductase-deficient tester strains were relatively resistant to these actions (4). These observations suggested to us that the residual mutagenicity observed at high levels of niridazole in the nitroreductase-deficient tester strain was due to residual enzyme activity and/or reflected the possible activity of a second nitroreductase with a different specificity for niridazole.

The purpose of the present study was to further define the role of bacterial nitroreductase in the mutagenicity of niridazole. We report herein that the mutagenicity of niridazole in the nitroreductase-deficient Salmonella tester strain described previously is due primarily to residual niridazole-specific nitroreductase activity but that a second nitroreductase activity also contributes to it.

MATERIALS AND METHODS

Salmonella typhimurium strain TA98 was obtained from Dr. B. N. Ames. Normally, derivatives lacking the "classical" nitroreductase (TA98NR) were isolated as niridazole resistant by plating overnight cultures on Columbia agar plates (BBL Microbiology Systems, Baltimore, Md.) containing niridazole (5 μg/ml). Niridazole-resistant colonies were recloned, and the retention of the deep-rough character was determined by sensitivity to crystal violet while the presence of the plasmid (pKM101) characteristic of TA98 was ascertained by ampicillin resistance (3). All selected mutant clones were then tested for mutagenic response to a panel of mutagens. These included 7-(bromo-methyl)-1,2-methylbenz(a)anthracene, 1-hydroxylaminoanthracene, 4-hydroxylaminoquinoline-1-oxide, 1,8-dinitropyrene, 2-aminoanthracene plus S-9, and 3,4-benzpyrene plus S-9. All chemicals were obtained from the Chemical Repository of the National Cancer Institute. Only mutants retaining full sensitivity to the mutagenicity of nonnitro chemicals were used for further studies.

Mutants completely devoid of the "classical" nitroreductases were isolated in the following manner. Discs containing the frame-shift mutagen 9-aminooacidine (200 μg) (2) were deposited on the surface of Columbia agar plates containing niridazole (3.3 or 10 μg/ml). The plates were incubated at 37° in the dark for 4 hr to permit diffusion of 9-aminooacidine from the discs, whereupon the discs were removed, and portions of overnight cultures of TA98 (10⁶ cells) were spread on the surfaces of the plates. The plates were incubated in the dark, and niridazole-resistant colonies were picked only from among the colonies growing near the zone of complete inhibition due to the 9-aminooacidine. These mutant clones were then processed as described above.

The mutagenicity assay was carried out as described by Ames et al. (3). No microsomal activation mixtures were used.

RESULTS AND DISCUSSION

The nitroreductase-deficient Salmonella tester strains utilized in previous studies were selected on the basis of resistance to nitrofurans and/or niridazole. These chemicals are known to induce base substitution mutations (11, 12, 26, 28, 29), and their use as selective agents favors the isolation of nitroreductase-deficient Salmonella arising as a result of base substitution mutations. Nitroreductase-deficient strains selected in this fashion may frequently possess a modified nitroreductase that differs from the parent "wild-type" enzyme by
we constructed a new set of nitroreductase-deficient tester strains by elevating concentrations of niridazole was due to such a modified enzyme, we constructed a new set of nitroreductase-deficient tester strains utilizing a frame-shift mutagen (e.g., 9-aminoacridine) as the mutagenic agent. Nitroreductase-deficient (niridazole-resistant) strains selected by this technique are expected to be devoid of residual niridazole nitroreductase activity because frame-shift mutations lead to premature chain termination and the generation of incomplete polypeptides rather than of a complete enzyme with a modified amino acid sequence.

The mutagenicity of niridazole differed markedly (Table 1) in the parent strain (TA98), the original nitroreductase-deficient strain (TA98NR201), and the 9-aminoacridine-induced nitroreductase-deficient strains (TA98NIR301, TA98NIR302, etc.). More specifically, we observed specific mutagenic activities of 2160 revertants/μg with the parent strain, 96 revertants/μg with the original TA98NR201 strain, and approximately 4 revertants/μg with strains presumably having nitroreductase deficiencies as the result of frame-shift mutations (0.2% of that observed in the parent strain). These results suggest that the mutagenicity observed in TA98NR201 in the presence of elevated levels of niridazole is due mainly (96%) to the residual activity inherent in the amino acid-substituted niridazole-specific nitroreductase (classical nitroreductase). The activity observed in the strains resulting from frame-shift mutations (e.g., 0.2% that of the parent strain) may well reflect the activity of a second nitroreductase ("nonclassical nitroreductase") which may be identical to the recently described nitroreductases for niridazole mutagenesis. Cancer Res., 40: 4599-4605, 1980.

REFERENCES


Table 1

Mutagenicity of niridazole in Salmonella strains deficient in niridazole-nitroreductase

<table>
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<tr>
<th>Niridazole (µg/plate)</th>
<th>TA98</th>
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Revertants/µg

| Revertants/µg | 2160 | 96  | 4.0 | 4.0 | 3.5 | 4.7 |

Our findings not only help define the role of bacterial nitroreductase in the mutagenicity of niridazole but further characterize the nitroreductase activity present in the Salmonella tester strain. These observations are timely in view of the recent findings that nitro compounds (e.g., nitropolycyclic aromatic hydrocarbons) may be ubiquitous in our environment as a result of mobile (diesel) and stationary combustion processes (5, 6, 8-10, 14-17, 24-27). The newly derived tester strains, entirely devoid of classical nitroreductase activity, may prove useful in evaluating the potential long-term hazards of nitroaromatic environmental pollutants, because they permit the study of the activity of these chemicals in a tester strain devoid of an enzyme activity that may be unique to bacteria (1, 13, 19-20). As such, they provide a means for the evaluation of the relative importance of bacterial and mammalian enzymes in chemical carcinogenesis and mutagenesis.

ACKNOWLEDGMENTS

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