AN ASSAY FOR THE DETECTION OF BACTERIAL DNA GYRASE INHIBITORS

MARCIA S. OSBURNE*, WILLIAM M. MAISE, and MICHAEL GREENSTEIN

American Cyanamid Company, Lederle Laboratories, Department of Microbial Genetics and Biochemistry, Pearl River, NY 10965, U.S.A.

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Bacterial topoisomerase II (DNA gyrase) is an essential enzyme that is known to be target of two major classes of antibiotics. The quinolones, synthetic products typified by nalidixic and oxolinic acids, inhibit the A subunit, probably by interfering with the DNA-rejoining step of the gyrase-mediated, DNA strand-passing reaction. The coumarins, natural products that inhibit gyrase, probably by competing with ATP for binding to the B subunit of the enzyme, include novobiocin, coumermycin A1, and derivatives. Here we describe an assay system which detects both classes of gyrase inhibitors in addition to the antibiotic cinodine, a novel inhibitor of bacterial DNA gyrase.

Materials and Methods

Bacterial Strains

_Bacillus subtilis_ strain DIN23 contains a chromosomal fusion of a DNA damage-inducible (din) promoter to the _Escherichia coli_ lacZ gene, and was obtained from R. YASBIN. The strain was derived from strain YB886 (metB5trpC2xin-lSPP-). We constructed a recM13 derivative of strain DIN23, i.e., DIN23_recM13, as previously described.

Fusion strains were resistant to erythromycin (1 μg/ml) and lincomycin (25 μg/ml) as a result of the Tn917 insertion.

The DIN Assay for the Detection of DNA Gyrase Inhibitors

_B. subtilis_ strains DIN23 and DIN23_recM13 were grown to late log phase (150~200 Klett units, Klett-Summerson colorimeter, green filter) in LB medium (0.5% tryptone, 0.5% yeast extract, 1% NaCl, pH 7.0) containing erythromycin and lincomycin. Two ml of culture were added to 40 ml DSM soft agar containing 4 ml of a 0.1% solution in 10% DMSO of 4-methylumbelliferyl-β-D-galactoside (MUG, Sigma). 15~30 minutes later, plates were inspected for fluorescence (an indication of β-galactosidase production) under long wave-length UV light. Plates were photographed with a Kodak 47B (blue) filter.

Results and Discussion

The DIN Assay

A number of DNA damaging agents and DNA gyrase inhibitors are known to induce an SOS-like response in _B. subtilis_. The relevant studies have made use of _B. subtilis_ strains which encode a din promoter fused to the lacZ gene of _E. coli_, and which, therefore, produce β-galactosidase when the SOS response is induced. Recent studies of the _B. subtilis_ recM13 mutation have indicated that recM13 mutants were poorly induced for the SOS response by both UV light and mitomycin C, whereas induction with the DNA gyrase subunit A inhibitor nalidixic acid appeared normal. Based on these results, we have developed an assay system which detects both classes of gyrase inhibitors in addition to the antibiotic cinodine, a novel inhibitor of bacterial DNA gyrase.

Fig. 1. β-Galactosidase induction in strains DIN23 (A) and DIN23_recM13 (B).

The assay procedure is described in the text. (1) nalidixic acid, 30 μg; (2) novobiocin, 30 μg; (3) mitomycin C, 500 ng; (4) novobiocin, 2 μg; (5) ethidium bromide, 10 μg; (6) and (7) chloramphenicol, 30 μg; (8) tetracycline, 10 μg; (9) ampicillin, 10 μg; (10) gentamicin, 10 μg; (11) methicillin, 5 μg; (12) cephalexin, 30 μg; (13) novobiocin, 30 μg; (14) nalidixic acid, 30 μg; (15) norfloxacin, 10 μg.

Current address: Procept, Inc., 840 Memorial Drive, Cambridge, MA 02139, U.S.A.
on these findings, we reasoned that many or all
gyrase inhibitors may induce an SOS response in
both the B. subtilis parent strain and its recM13
derivative, whereas other antibacterial agents may
induce only one or neither of the two strains.
Therefore strains DIN23 and DIN23-recM13 were
assayed for their ability to produce β-galactosidase
in response to treatment with various drugs. A
typical assay plate (described in Materials and
Methods) is shown in Fig. 1. The presence or absence
of fluorescence is sometimes difficult to ascertain
from a photograph, but can be easily verified by
direct observation of assay plates.
SOS induction by a wide variety of compounds
was tested in this assay system. These included
DNA gyrase A and B inhibitors, antitumor antibio-
tics and other DNA-binding compounds, antimeta-
bolites, cell wall or cell membrane inhibitors,
RNA polymerase inhibitors, protein synthesis in-
hibitors, and polyether antibiotics. Although some
of the compounds induced one or the other or
neither of the other or neither of the two strains,
only inhibitors of DNA gyrase subunit A or B in-
duced both strains (Table 1). This finding led to
the use of the DIN assay to detect potential in-
hibitors of DNA gyrase, in that any compound
that induces both of these strains is suspected to
inhibit DNA gyrase.

That the recM13 strain was induced by
compounds which do not normally induce an SOS
response was an interesting and unexpected result.
The altered SOS regulation conferred by the recM13
mutation is discussed elsewhere. The mutation
appears to confer a low-level constitutive SOS
response, and, since the mutation also confers
increased sensitivity to DNA damaging agents such
as mitomycin C, such drugs may kill the mutant
strain before it can mount an SOS response.

Table 1. Induction of β-galactosidase in strains DIN23 and DIN23-recM13 by various
drugs.

<table>
<thead>
<tr>
<th>Category</th>
<th>Drug</th>
<th>DIN23</th>
<th>DIN23-recM13</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. No induction</td>
<td>Actinomycin*e</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>Granaticine*e</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>5-Fluorouracil*d</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>Bacitracin*f</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Benzylpenicillin*f</td>
<td>–</td>
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<td></td>
<td>Polymyxin B*f</td>
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<tr>
<td></td>
<td>Heliomycin*e</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Rifampicin*e</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>B. DIN23 induction</td>
<td>Bleomycin*e</td>
<td>+ (50 ng)*k</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Mitomycin C*e</td>
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<td>–</td>
</tr>
<tr>
<td>C. DIN23-recM13 induction</td>
<td>Adriamycin*e</td>
<td>–</td>
<td>+ (1 μg)*k</td>
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<tr>
<td></td>
<td>Ethidium bromide*e</td>
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<td>+ (5 μg)*k</td>
</tr>
<tr>
<td></td>
<td>Mitoxantrone*e</td>
<td>–</td>
<td>+ (1 μg)*k</td>
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<tr>
<td></td>
<td>Netropsin*f</td>
<td>–</td>
<td>+ (1 μg)*k</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol*e</td>
<td>–</td>
<td>+ (10 μg)*k</td>
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<tr>
<td></td>
<td>Gentamicin*e</td>
<td>–</td>
<td>+ (50 μg)*k</td>
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<tr>
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<td>Tetracycline*e</td>
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<td></td>
<td>Ampicillin*f</td>
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<td>+ (1 μg)*k</td>
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<tr>
<td></td>
<td>Cephalothin*f</td>
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</tr>
<tr>
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<td>Methicillin*f</td>
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</tr>
<tr>
<td></td>
<td>Vancomycin*e</td>
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<td>+ (30 μg)*k</td>
</tr>
<tr>
<td></td>
<td>Monensin*b</td>
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<td>+ (0.5 μg)*k</td>
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<tr>
<td>D. Both strains induced</td>
<td>Nalidixic acid*e</td>
<td>+ (1 μg)*k</td>
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<td></td>
<td>Norfloxacin*e</td>
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<td>+ (0.05 μg)*k</td>
</tr>
<tr>
<td></td>
<td>Novobiocin*b</td>
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<td>+ (0.05 μg)*k</td>
</tr>
<tr>
<td></td>
<td>Cinodine*</td>
<td>+ (0.5 μg)*k</td>
<td>+ (0.5 μg)*k</td>
</tr>
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</table>

*a Inhibitors of DNA gyrase subunit A. b Inhibitors of DNA gyrase subunit B. c Antitumor antibiotics and other DNA-binding compounds. d Antimetabolites. e Protein synthesis inhibitors. f Cell wall or membrane inhibitors. g RNA synthesis inhibitors. h Polyether antibiotics. i DNA gyrase inhibitor, subunit not determined. j β-Galactosidase induction, scored as a “+” or a “−” was determined by means of the plate induction assay. Drugs were applied to assay plates in 1 ~ 20 μl volumes. k Lowest amount of drug which induced β-galactosidase.
Detection of a Novel DNA Gyrase Inhibitor Using the DIN Assay

Cinodine, a glyccocinamoylspermidine antibiotic originally characterized as an inhibitor of DNA synthesis\(^1\), was positive in the DIN assay. This compound was subsequently tested \textit{in vitro} and was found to inhibit bacterial DNA gyrase activity\(^2\).\(^3\).\(^4\).

Summary

In summary, we have developed a sensitive detection system for inhibitors of bacterial DNA gyrase. The use of \textit{B. subtilis} as the host organism confers the advantage that it is sensitive to both gyrase subunit A and B inhibitors, whereas \textit{E. coli} is relatively insensitive to B subunit inhibitors \textit{in vivo}. Using this assay, we identified a new DNA gyrase inhibitor with a novel structure.

Acknowledgments

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References

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