AN ASSAY FOR THE DETECTION OF BACTERIAL DNA GYRASE INHIBITORS

MARCIA S. OSBURNE*,†, WILLIAM M. MAIESE, and MICHAEL GREENSTEIN

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

(Received for publication June 7, 1993)

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[1~4]. The coumarins, natural products that inhibit gyrase, probably by competing with ATP for binding to the B subunit of the enzyme[5,6], 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[7].

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[8], and was obtained from R. YASBIN. The strain was derived from strain YB886 (*metB5trpC2xin-1SPβ-*). We constructed a *recM13* derivative of strain DIN23, *i.e.*, DIN23*recM13*, as previously described[9]. Fusion strains were resistant to erythromycin (1 μg/ml) and lincomycin (25 μg/ml) as a result of the Tn*917* insertion.

The DIN Assay for the Detection of DNA Gyrase Inhibitors

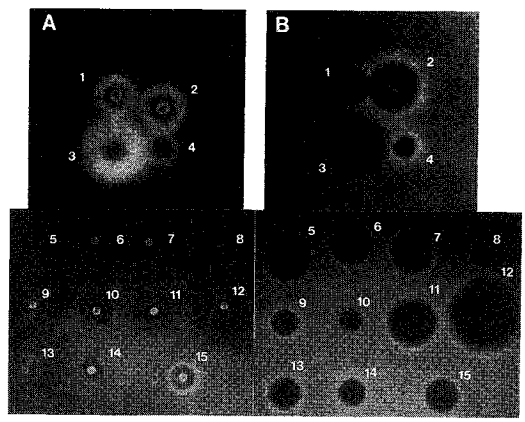
B. subtilis strains DIN23 and DIN23*recM13* were grown to late log phase (150~200 Klett units, Klett-Summerson colorimeter, green filter) in LB medium[10] containing erythromycin and lincomycin. Two ml of culture were added to 40 ml DSM[11] soft agar, which was then poured onto a 22.9× 22.9 cm assay plate containing DSM agar. Compounds to be tested were spotted onto each plate. Following overnight growth at 30°C for the DIN23 strain and 37°C for the DIN23*recM13* strain, the plates were overlayed with 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*[8,9,12]. 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[9,13]. Based

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) cephalothin, 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 antibiotics and other DNA-binding compounds, antimetabolites, cell wall or cell membrane inhibitors, RNA polymerase inhibitors, protein synthesis inhibitors, 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 induced both strains (Table 1). This finding led to the use of the DIN assay to detect potential inhibitors 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[9]. 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[j] in strains DIN23 and DIN23*recM13* by various drugs.

Category	Drug	DIN23	DIN23*recM13*
A. No induction	Actinomycin[c]	–	–
	Granaticin[c]	–	–
	5-Fluorouracil[d]	–	–
	Bacitracin[f]	–	–
	Benzylpenicillin[f]	–	–
	Polymyxin B[f]	–	–
	Heliomycin[g]	–	–
	Rifampicin[g]	–	–
B. DIN23 induction	Bleomycin[c]	+ (50 ng)[k]	–
	Mitomycin C[c]	+ (1 ng)[k]	–
C. DIN23*recM13* induction	Adriamycin[c]	–	+ (1 μg)[k]
	Ethidium bromide[c]	–	+ (5 μg)[k]
	Mitoxantrone[c]	–	+ (1 μg)[k]
	Netropsin[c]	–	+ (1 μg)[k]
	Chloramphenicol[e]	–	+ (10 μg)[k]
	Gentamicin[e]	–	+ (50 μg)[k]
	Tetracycline[e]	–	+ (10 μg)[k]
	Ampicillin[f]	–	+ (1 μg)[k]
	Cephalothin[f]	–	+ (0.5 μg)[k]
	Methicillin[f]	–	+ (5 μg)[k]
	Vancomycin[f]	–	+ (30 μg)[k]
	Monensin[h]	–	+ (0.5 μg)[k]
D. Both strains induced	Nalidixic acid[a]	+ (1 μg)[k]	+ (1 μg)[k]
	Norfloxacin[a]	+ (0.1 μg)[k]	+ (0.05 μg)[k]
	Novobiocin[b]	+ (0.5 μg)[k]	+ (0.05 μg)[k]
	Cinodine[i]	+ (0.5 μg)[k]	+ (0.5 μg)[k]

[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 glycocinnamoylspermidine antibiotic originally characterized as an inhibitor of DNA synthesis[14], was positive in the DIN assay. This compound was subsequently tested *in vitro* and was found to inhibit bacterial DNA gyrase activity[7,15].

Summary

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

Acknowledgments

We are grateful to R. YASBIN for bacterial strains and helpful discussions.

References

1) COZZARELLI, N. R.: DNA gyrase and the supercoiling of DNA. Science 207: 953~960, 1980
2) GELLERT, M.: DNA topoisomerases. Annu. Rev. Biochem. 50: 879~910, 1981
3) GELLERT, M.; K. MIZUUCHI, M. H. O'DEA, T. ITOH & J. TOMIZAWA: Nalidixic acid resistance: a second genetic character involved in DNA gyrase activity. Proc. Natl. Acad. Sci. U.S.A. 74: 4772~4776, 1977
4) SUGINO, A.; C. PEEBLES, K. KREUZER & N. COZZARELLI: Mechanism of action of nalidixic acid: purification of *Escherichia coli nalA* gene product and its relationship to DNA gyrase, a novel nicking-closing enzyme. Proc. Natl. Acad. Sci. U.S.A. 74: 4767~4771, 1977
5) GELLERT, M.; M. H. O'DEA, T. ITOH & J. TOMIZAWA: Novobiocin and coumermycin inhibit DNA supercoiling catalyzed by DNA gyrase. Proc. Natl. Acad. Sci. U.S.A. 73: 4474~4478, 1976
6) SUGINO, A.; N. HIGGINS, P. BROWN, C. PEEBLES & N. COZZARELLI: Energy coupling in DNA gyrase and the mechanism of action of novobiocin. Proc. Natl. Acad. Sci. U.S.A. 75: 4838~4842, 1978
7) OSBURNE, M. S.; W. M. MAIESE & M. GREENSTEIN: In vitro inhibition of bacterial DNA gyrase by cinodine, a glycocinnamoylspermidine antibiotic. Antimicrob. Agents Chemother. 34: 1450~1452, 1990
8) LOVE, P. E.; M. J. LYLE & R. E. YASBIN: DNA damage-inducible (*din*) loci are transcriptionally activated in competent *Bacillus subtilis*. Proc. Natl. Acad. Sci. U.S.A. 82: 6201~6205, 1985
9) OSBURNE, M. S.; S. M. ZAVODNY, M. GREENSTEIN & W. M. MAIESE: Phenotypes conferred by the *Bacillus subtilis recM13* mutation and the *din23* fusion. Mutat. Res., DNA Repair 274: 79~84, 1992
10) MILLER, J. H. (*Ed.*): Experiments in Molecular Genetics., p. 433, Cold Spring Harbor Laboratory, 1972
11) SONENSHEIN, A. L.; B. CAMI, J. BREVET & R. COTE: Isolation and characterization of rifampin-resistant and streptolydigen-resistant mutants of *Bacillus subtilis* with altered sporulation properties. J. Bacteriol. 120: 253~265, 1974
12) OSBURNE, M. S.; S. M. ZAVODNY & G. A. PETERSON: Drug-induced relaxation of supercoiled plasmid DNA in *Bacillus subtilis* and induction of the SOS response. J. Bacteriol. 170: 442~445, 1988
13) LOVETT, C. M., Jr.; P. E. LOVE, R. E. YASBIN & J. W. ROBERTS: SOS-like induction in *Bacillus subtilis*: induction of the RecA protein analog and a damage-inducible operon by DNA damage in Rec+ and DNA repair-deficient strains. J. Bacteriol. 170: 1467~1474, 1988
14) GREENSTEIN, M.; J. L. SPETH & W. M. MAIESE: Mechanism of action of cinodine, a glycocinnamoylspermidine antibiotic. Antimicrob. Agents Chemother. 20: 425~432, 1981
15) NORCIA, M.; L. WONDRACK & J. SUTCLIFFE: Cinodine has a unique mechanism of DNA gyrase inhibition. Abstract of 91st Annu. Meet. Am. Soc. Microbiol, No. A94, p 16, Dallas, May 5~9, 1991