Effects of DNA Gyrase Inhibitors in *Escherichia coli* Topoisomerase I Mutants

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Relaxation of titratable supercoils in bacterial nucleoids was measured following treatment of *topA* mutants with coumermycin or oxolinic acid, inhibitors of DNA gyrase. Relaxation occurred after treatment of the mutants with either inhibitor. We detected no significant difference in relaxation between *topA*⁻ and *topA*⁺ strains treated with coumermycin. This finding, together with previous observations, supports the idea that relaxation caused by coumermycin probably arises from the relaxing activity of gyrase itself. The source of DNA relaxation caused by oxolinic acid was not identified. Nucleoid supercoiling can be increased by adding oxolinic acid to a strain that carries three topoisomerase mutations: $\Delta topA$, *gyrB225*, and *gyrA* (Nal^r) (S. H. Manes, G. J. Pruss, and K. Drlica, J. Bacteriol. 155:420-423, 1983). We found that this increase in supercoiling requires partial sensitivity to the drug and at least the $\Delta topA$ and *gyrA* mutations. Full resistance to oxolinic acid in the presence of the $\Delta topA$, *gyrB225*, and *gyrA* mutations was conferred by an additional mutation that maps at or near *gyrB*.

One of the striking features of the bacterial chromosome is that its DNA is under negative superhelical tension (21, 31). Since fluctuations in supercoiling can have profound effects on gene expression both in vitro and in vivo (for reviews, see references 1, 5, 7, 13), it is important to understand how the level of supercoiling is controlled. Several enzymatic activities have been found that can alter DNA supercoiling: DNA gyrase can both introduce and remove negative supercoils (10, 11), and DNA topoisomerases I (30) and III (3) can remove them. At least one of the mechanisms for the control of supercoiling appears to be a supercoiling-dependent regulation of the genes encoding gyrase and topoisomerase I: when supercoiling levels decrease, gyrase expression increases (8, 14) and topoisomerase I expression decreases (27).

The availability of mutations in the genes encoding gyrase and topoisomerase I and of specific inhibitors of gyrase has allowed the physiological study of DNA supercoiling. The A subunit of gyrase is inhibited by oxolinic acid, nalidixic acid, and related quinolone antibiotics (9, 25), and the B subunit is inhibited by coumarin antibiotics such as coumermycin and novobiocin (10). Members of either class of drug are capable of blocking the introduction of titratable supercoils into bacteriophage lambda during superinfection of a lysogen (9, 10). In addition, the drugs, as well as the incubation of temperature-sensitive gyrase mutants at a nonpermissive temperature, cause relaxation of titratable supercoils from the bacterial chromosome (6, 12, 22, 28). Together, these results indicate that gyrase is responsible for introducing and maintaining superhelical tension in the chromosome. Mutations in *topA*, the gene encoding topoisomerase I (23, 26), cause bacterial chromosomes and plasmid DNA to have higher-than-normal levels of titratable supercoiling (17, 18, 20). This observation suggests that topoisomerase I modulates the effect of gyrase by removing supercoils. Such an inference is supported by the additional observation that

many *topA* mutants acquire mutations in the genes encoding subunits of gyrase (4, 18-20). These mutations seem to compensate for the loss of topoisomerase I, and some have been shown to reduce gyrase activity (8, 19).

Inhibitors of gyrase can either increase or decrease titratable DNA supercoiling, depending on the inhibitor concentration and on the genetic background of the bacterial cells treated with the inhibitor (12). To examine the contribution of topoisomerase I to these changes in supercoiling, we measured the effect of gyrase inhibitors on titratable chromosomal DNA supercoiling in strains carrying *topA* mutations. We found that the absence of topoisomerase I had little effect on the ability of inhibitors of gyrase to relax DNA. However, in a strain carrying mutations in both *gyrA* and *gyrB*, the absence of topoisomerase I appeared to be necessary for oxolinic acid to increase supercoiling.

MATERIALS AND METHODS

Chemicals, reagents, and bacterial strains. [*methyl*-³H]thymidine (40 to 60 Ci/mmol) was purchased from New England Nuclear Corp., Boston, Mass. Ethidium bromide and nalidixic acid were purchased from Sigma Chemical Co., St. Louis, Mo. Oxolinic acid and coumermycin were gifts from Warner-Lambert Research Institute, Morris Plains, N.J., and Bristol Laboratories, Syracuse, N.Y., respectively.

All strains used are derivatives of *Escherichia coli* K-12. The relevant genotypes of these strains are listed in Table 1. For nucleoid isolations and DNA synthesis measurements, strains were grown in M9 minimal medium (15) supplemented with Casamino Acids (0.5%) and cysteine (50 µg/ml); uracil (30 µg/ml) and tryptophan (40 µg/ml) were added as required. P1-mediated transduction was performed by the method of Wall and Harriman (29). Nalidixic acid-resistant transductants were selected and screened on LB agar plates (15) containing nalidixic acid at various concentrations. Tetracycline-resistant transductants were selected and screened on LB agar plates containing tetracycline (20 µg/ml) plus nalidixic acid at various concentrations. All LB media used for growing strain DM800 and its derivatives

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TABLE 1. Bacterial strains

Strain	Relevant genotype	Source or reference
DM4100	<i>topA</i> ⁺	23
DM700	$\Delta(\textit{topA cysB})217$	4, 23
DM800	<i>gyrB225</i> $\Delta(\textit{topA cysB})204$	4, 23
KD200	RF1 <i>gyrB225 tna::Tn10</i>	This laboratory (see text)
GP200	DM800 <i>gyrA</i> (Nal ^r)	This laboratory by P1 transduction of Nal ^r from LL307
GP201	DM4100 <i>gyrA</i> (Nal ^r)	This laboratory by P1 transduction of Nal ^r from GP200
GP202	SD104-20 <i>gyrA</i> (Nal ^r)	This laboratory by P1 transduction of Nal ^r from LL307
GP203	SD104-20 $\Delta(\textit{topA cysB})204$ <i>gyrA</i> (Nal ^r)	This laboratory by P1 transduction of $\Delta(\textit{topA cysB})204$ from DM800 and Nal ^r from LL307
LL307	<i>gyrA</i> (Nal ^r)	L. Lindahl, University of Rochester, Rochester, N.Y.
RF1	GP200 <i>gyrB500</i> (Nal ^r)	This laboratory (see text)
SD104-20	DM4100 <i>gyrB225 tna::Tn10</i>	4
SD108	DM800 <i>topA</i> ⁺ <i>cysB</i> ⁺	4

contained L-cysteine (40 $\mu\text{g/ml}$). Temperature-sensitive strains were grown at 30°C, and thermal sensitivity was screened by incubation of plates at 42°C.

Preparation and sedimentation of bacterial nucleoids. Cells were radioactively labeled by growth in 8 to 10 μCi of [*methyl*-³H]thymidine per ml for 40 to 45 min prior to treatment with coumermycin (50 $\mu\text{g/ml}$) or oxolinic acid (2 $\mu\text{g/ml}$). Cell lysis was performed by the method of Stonington and Pettijohn (24) as modified by Worcel and Burgi (31) to obtain membrane-free nucleoids; all lysates cleared within 1.5 to 5 min at 20 to 22°C. Samples of whole cell lysates (about 0.5 μg of DNA) were loaded directly onto ethidium bromide-containing sucrose density gradients for sedimentation measurements as described previously (6). ¹⁴C-labeled bacteriophage T4B was included in each gradient as a sedimentation marker. Fractions were collected from the bottom of each gradient directly into vials, and radioactivity was measured with Triton X-100-containing scintillation fluid. The means of the radioactivity distributions in the ³H-labeled chromosome and ¹⁴C-labeled bacteriophage T4B peaks were used to determine relative sedimentation rates. An average sedimentation coefficient was then calculated by using *S* = 1025 for T4B (2).

Measurement of DNA synthesis rate. Cells (200- μl aliquots) were pulse-labeled with 2.5 μCi of [*methyl*-³H]thymidine per ml for 2 min. Incorporation was terminated by the addition of 5 volumes of cold 5% (wt/vol) trichloroacetic acid. Rates of incorporation were calculated from determinations of acid-precipitable radioactivity.

RESULTS

Relaxation of chromosomal DNA in *topA* mutants by coumermycin. Nucleoids isolated from wild-type cells treated with coumermycin have a lower level of titratable

supercoiling than those isolated from untreated cells (6). To measure DNA relaxation caused by coumermycin in *topA* mutants, we treated two mutant strains with the drug, lysed the cells, and determined nucleoid sedimentation rates in the presence of various concentrations of ethidium bromide. Figure 1 shows the results obtained with strain RS2, which contains the *topA10* point mutation, and strain DM700, which contains a deletion of *topA*. In both cases, coumermycin treatment resulted in the relaxation of titratable supercoiling. We also measured titratable supercoiling at various times after the addition of coumermycin to the *topA* deletion strain DM800 and its *topA*⁺ derivative SD108. We observed little difference in the kinetics of relaxation (Fig. 2). Taken together, these results indicate that topoisomerase I has little effect on DNA relaxation by coumermycin.

Relaxation of chromosomal DNA in a *topA* mutant by oxolinic acid. Nucleoids isolated from wild-type cells also exhibit a decrease in titratable supercoiling when the cells are treated with sufficient concentrations of oxolinic acid (12). Therefore, we treated strain RS2 (*topA10*) with oxolinic acid and measured nucleoid supercoiling. The concentration of ethidium bromide required to titrate the negative supercoils in chromosomal DNA decreased when the nucleoids were obtained from cells treated with oxolinic acid (Fig. 3). Thus, a mutation in *topA* failed to block the relaxation of chromosomal DNA induced by oxolinic acid.

Increase of supercoiling in a *topA* deletion mutant carrying mutations in both *gyrase* genes by oxolinic acid. In the course of studying the effects of oxolinic acid on supercoiling in *topA* mutants, we constructed a *gyrA* (Nal^r) derivative of the *topA* deletion mutant DM800. This nalidixic acid-resistant strain (GP200) contains three topoisomerase mutations: *gyrA* (Nal^r), *gyrB225*, and $\Delta\textit{topA}$. We were surprised to find that nucleoids isolated from strain GP200 treated with oxolinic acid were more negatively supercoiled than nucleoids isolated from the untreated control (Fig. 4A and reference 12). The *gyrA* (Nal^r) mutation is apparently required for this oxolinic acid-induced increase in supercoiling; treatment of the drug-sensitive parental strain (DM800) with oxolinic acid did not increase supercoiling (Fig. 4B).

To more clearly define the genetic factors involved in the oxolinic acid-induced increase in supercoiling, we examined the effect of oxolinic acid on the supercoiling of strains carrying the above three topoisomerase mutations singly or in combination. None of these strains had previously contained a *topA* deletion and were, therefore, unlikely to contain unidentified compensatory mutations. Oxolinic acid treatment of strains carrying only the *gyrA* (Nal^r) mutation or only the *gyrB225* compensatory mutation failed to increase nucleoid supercoiling (data not shown). Similarly, oxolinic acid treatment of a strain (GP202) constructed by transducing *gyrA* (Nal^r) into strain SD104-20 (*gyrB225*) also failed to increase nucleoid supercoiling (Fig. 4C). Thus, the *gyrA* (Nal^r) allele and the *gyrB225* allele are insufficient to elicit oxolinic acid-induced increases in supercoiling. In contrast, oxolinic acid treatment of a strain (GP203) constructed by transducing both *gyrA* (Nal^r) and $\Delta\textit{topA}$ into strain SD104-20 (*gyrB225*) increased nucleoid supercoiling (Fig. 4D). These experiments suggest that both the *gyrA* (Nal^r) and $\Delta\textit{topA}$ mutations are required for oxolinic acid to increase DNA supercoiling. We did not directly test whether the *gyrB225* mutation is also required because it is difficult to transduce $\Delta\textit{topA}$ into a strain lacking a compensatory mutation without also obtaining spontaneous compensatory mutations (4).

FIG. 1. Relaxation of chromosomal DNA by coumermycin in *topA* mutants. (A) ^3H -labeled nucleoids from *E. coli* K-12 strain RS2 (*topA10*) treated with 0 (○) or 50 (●) μg of coumermycin per ml for 35 min were sedimented into sucrose gradients containing the indicated concentrations of ethidium bromide. Each gradient contained ^{14}C -labeled bacteriophage T4B as a sedimentation marker. The nucleoid sedimentation rates were determined from the relative distances sedimented by nucleoid and marker peaks. (B) Sedimentation rates of ^3H -labeled nucleoids from *E. coli* K-12 strain DM700 (ΔtopA) treated with 0 (○) or 50 (●) μg of coumermycin per ml for 35 min were determined as described for panel A.

Correlation of increased supercoiling induced by oxolinic acid with partial sensitivity to the drug. The triple topoisomerase mutant GP200 (ΔtopA *gyrB225* *gyrA* [Nal^r]) was constructed by transducing a *gyrA* (Nal^r) allele into strain DM800 (ΔtopA *gyrB225*). Nalidixic acid-resistant transductants were selected on agar plates containing 5 μg of nalidixic acid per ml and were tested for growth on agar plates containing 20 μg of nalidixic acid per ml, the standard drug concentration for selecting *gyrA* (Nal^r) mutants (15). All transductants grew at the higher drug concentration; however, we found that strain GP200, a randomly chosen, nalidixic acid-resistant transductant, failed to grow on agar plates containing 50 μg of nalidixic acid per ml, a drug concentration permissive for growth of the Nal^r donor strain LL307. Transduction of the *gyrA* (Nal^r) allele from GP200 into the closely related wild-type strain DM4100 produced a strain (GP201) that grew on plates containing 50 μg of nalidixic acid per ml. Thus, in the DM800 background, the *gyrA* allele behaved anomalously: cells were partially sensitive to oxolinic acid. The *gyrA* allele from LL307 also behaved anomalously in strain GP203, which contains the same topoisomerase mutations as strain GP200 but in a wild-type background to eliminate unidentified mutations. GP203 was resistant to 20 but not 50 μg of nalidixic acid per ml and exhibited an oxolinic acid-induced increase in supercoiling (Fig. 4D) such as that observed in GP200 (Fig.

4A). The partial sensitivity of strains GP200 and GP203 to oxolinic acid was also observed by measuring DNA synthesis rates after the addition of the drug to exponentially growing cultures (Fig. 5). DNA synthesis in both GP200 and GP203 was partially inhibited by drug concentrations that rapidly inhibited synthesis in strains with wild-type *gyrA* alleles but had little effect on strains with *gyrA* (Nal^r) alleles in *topA*⁺ backgrounds.

The effects of oxolinic and nalidixic acids on strains related to DM800 are summarized in Table 2. Only strains that were partially sensitive to oxolinic acid exhibited the drug-induced increase in titratable supercoiling. The two strains in this category have a deletion of *topA*, a defective *gyrB* allele (4, 8, 18) that lowers titratable supercoiling in an otherwise wild-type background (18), and a *gyrA* (Nal^r) allele that confers resistance to oxolinic and nalidixic acids in an otherwise wild-type background.

Abolition of partial sensitivity to oxolinic acid by a mutation at or near *gyrB*. The partial sensitivity of GP200 to nalidixic acid allowed us to select fully resistant mutants by growing the strain on agar containing 50 μg of nalidixic acid per ml. One mutant obtained in this fashion (RF1) was characterized further. When strain RF1 was treated with oxolinic acid, supercoiling of nucleoids (Fig. 4E) and DNA synthesis (Fig. 5) were unaffected, confirming that the strain is fully resistant to inhibitors of the A subunit of gyrase. The observation

FIG. 2. Kinetics of DNA relaxation by coumermycin in *topA*⁺ and *topA*⁻ strains. Sedimentation rates of ³H-labeled nucleoids from *E. coli* K-12 strains SD108 (●) and DM800 (○) treated with 50 μg of coumermycin per ml for the times indicated were determined at a variety of ethidium bromide concentrations as described in the legend to Fig. 1A. For each treatment time, the ethidium bromide concentration required to titrate the supercoils and generate a sedimentation minimum was determined from plots similar to those shown in Fig. 1.

that RF1 did not exhibit an increase in nucleoid supercoiling in response to oxolinic acid further supports the idea that partial sensitivity to the drug is required for that phenomenon, since full resistance eliminates it.

Preliminary mapping experiments indicated that the mutation conferring resistance to 50 μg of nalidixic acid per ml in strain RF1 is not linked to *gyrA*; therefore, we checked for linkage to *gyrB*. Two complementary transduction experiments indicated that the mutation maps at or near *gyrB*.

The first experiment was done to determine whether replacement of the *gyrB* allele in RF1 with a different *gyrB* allele eliminates resistance to 50 μg of nalidixic acid per ml. As the replacement, we used a *gyrB* allele (formerly known as *gyrB41*) which carries two mutations: *gyrB203*, which confers temperature sensitivity, and *gyrB221*, which confers resistance to coumermycin (14). A strain carrying tetracycline resistance (*tna::Tn10*) closely linked to this mutant *gyrB* allele was the donor for the transduction. We selected tetracycline-resistant transductants and screened for temperature sensitivity and resistance to different concentrations of nalidixic acid. The cotransduction frequency of tetracycline resistance and temperature sensitivity was 93% (888 of 958). However, only 4% (28 of 678) of the temperature-sensitive transductants tested retained resistance to 50 μg of nalidixic acid per ml (all transductants remained resistant to 20 μg of nalidixic acid per ml). Thus, the replacement of the *gyrB* allele in strain RF1 eliminates resistance to 50 μg of nalidixic acid per ml.

A small fraction (7%) of the tetracycline-resistant transductants were temperature resistant. Of these, 97% (67 of 70) were resistant to 50 μg of nalidixic acid per ml; they represent transductants which received *tna::Tn10* but not the *gyrB* allele from the donor and therefore have *tna::Tn10* closely linked to the *gyrB* allele of RF1. Four of these transductants, KD200-1, KD200-2, KD200-3, and KD200-4, served as donors for the second transduction experiment to determine whether resistance to 50 μg of nalidixic acid per ml is cotransducible with the *gyrB* allele of RF1 into a recipient (GP200) which is resistant to only 20 μg of nalidixic acid per ml. Again, we selected tetracycline-resistant transductants and screened for resistance to different concentrations of nalidixic acid. Sixty percent (110 of 186) of the tetracycline-resistant transductants formed colonies on agar containing 50 μg of nalidixic acid per ml. Thus, an allele from RF1 that is closely linked to *gyrB* confers resistance to 50 μg of nalidixic acid per ml when introduced into strain GP200. Resistance to 50 μg of nalidixic acid per ml cotransduced with resistance to tetracycline at a lower frequency in the second experiment than in the first. This difference probably arose from scoring uncertainties: colonies with intermediate phenotypes (slow growth on plates containing 50 μg of nalidixic acid per ml) were considered sensitive to that concentration of the drug.

Once we learned that resistance to 50 μg of nalidixic acid per ml maps at or near *gyrB*, we were able to determine if the mutation by itself confers resistance to the drug. It does not. We transduced *tna::Tn10* from KD200, a *tna::Tn10*-containing, temperature-resistant derivative of RF1, into the

FIG. 3. Relaxation of chromosomal DNA by oxolinic acid in a *topA10* mutant. Sedimentation rates of ³H-labeled nucleoids from *E. coli* K-12 strain RS2 (*topA10*) treated with 0 (○) or 2 (●) μg of oxolinic acid per ml for 10 min were determined as described in the legend to Fig. 1A.

FIG. 4. Effect of oxolinic acid on the sedimentation of nucleoids from topoisomerase mutants. Sedimentation rates of ^3H -labeled nucleoids from *E. coli* cells treated with 0 (○) or 2 (●) μg of oxolinic acid per ml for 10 min were determined as described in the legend to Fig. 1A. (A) Strain GP200; (B) strain DM800; (C) strain GP202; (D) strain GP203; (E) strain RF1.

wild-type strain DM4100. None of 200 tetracycline-resistant transductants were resistant to even 4 μg of nalidixic acid per ml.

In the first transduction experiment described above, we also scored cotransduction of novobiocin resistance with *tna::Tn10*. When the *gyrB221* and *gyrB203* mutations were transduced into wild-type strains, novobiocin resistance and temperature sensitivity both showed about 90% cotransduction with *tna::Tn10*. However, when we attempted to transduce these two *gyrB* mutations into strain DM800, we found that cotransduction of novobiocin resistance and tetracycline resistance was very low, although temperature sensitivity and tetracycline resistance showed the expected linkage. In the same experiment, we found no (0 of 750) tetracycline-resistant transductants which were also novobiocin resistant. R. Menzel (personal communication) has found that temperature-sensitive *gyrB203 gyrB221* transductants of DM800 exhibit coumermycin resistance after the

deletion of *topA* is replaced with *topA*⁺. Apparently, the *gyrB221* mutation (Cou^r) is suppressed in DM800.

DISCUSSION

Treatment of *E. coli* cells with inhibitors of gyrase can lower or raise titratable supercoiling of chromosomal DNA (12), and in the experiments described above we examined the effects of *topA* mutations on these changes in supercoiling. We found that the *topA* gene is not required for coumermycin to lower titratable DNA supercoiling in bacterial nucleoids (Fig. 1 and 2). Neither the rate nor the extent of relaxation by coumermycin appears to be affected by the absence of topoisomerase I (Fig. 2); thus, it seems unlikely that topoisomerase I contributes significantly to this process in wild-type cells. Since coumermycin does not inhibit the relaxing activity of gyrase in vitro (10), it is likely that gyrase itself relaxes chromosomal DNA when *topA* mutants or wild-type strains are treated with coumermycin.

TABLE 2. Effect of oxolinic and nalidixic acids on cell growth, DNA synthesis, and DNA supercoiling in topoisomerase mutants

Strain	Relevant genotype	Growth ^a	Inhibition of DNA synthesis ^b	Increase in DNA supercoiling ^c
DM4100	Wild type	—	+	—
DM800	<i>gyrB225 topA</i>	—	+	—
GP200	<i>gyrA</i> (Nal ^r) <i>gyrB225</i> Δ <i>topA</i>	±	±	+
GP201	<i>gyrA</i> (Nal ^r)	+	—	— ^d
RF1	<i>gyrA</i> (Nal ^r) <i>gyrB225 gyrB500</i> Δ <i>topA</i>	+	—	—
SD104-20	<i>gyrB225</i>	—	+	— ^d
GP202	<i>gyrA</i> (Nal ^r) <i>gyrB225</i>	+	—	—
GP203	<i>gyrA</i> (Nal ^r) <i>gyrB225</i> Δ <i>topA</i>	±	±	+

^a Growth was tested on agar plates in the presence of 20 or 50 μg of nalidixic acid per ml; +, growth at 50 $\mu\text{g}/\text{ml}$; ±, growth at 20 but not 50 $\mu\text{g}/\text{ml}$; —, no growth at either concentration.

^b Data are taken from Fig. 5. Relative to untreated controls, + indicates that DNA synthesis rates dropped to less than 10% following treatment with oxolinic acid; — indicates that the rates dropped to greater than 90% of untreated control rates; and ± indicates that the rates dropped to about 50% of untreated control rates.

^c Data are taken from Fig. 4; + indicates that titratable chromosomal supercoiling following oxolinic acid treatment was greater than that measured in the untreated controls, and — indicates that oxolinic acid caused no change or a decrease in supercoiling.

^d Data not shown.

FIG. 5. Inhibition of DNA synthesis by oxolinic acid in topoisomerase mutants. The incorporation of [3 H]thymidine during a 2-min pulse was measured during incubation of *E. coli* cells with 0 (open symbols) or 2 (solid symbols) μ g of oxolinic acid per ml to estimate the rate of DNA synthesis. Incorporation at the time of drug addition is taken as 100%. (A) Strain DM4100 (∇ and $\blacktriangledown$), strain DM800 ($\circ$ and $\bullet$), strain GP200 ($\square$ and $\blacksquare$), strain GP201 ($\triangle$ and $\blacktriangle$), and strain RF1 ($\diamond$ and $\blacklozenge$); (B) strain SD104-20 ($\triangle$ and $\blacktriangle$), strain GP202 ($\circ$ and $\bullet$), and strain GP203 ($\square$ and $\blacksquare$).

At high concentrations, oxolinic acid also causes chromosomal DNA relaxation in wild-type cells (12) and in *topA* mutants (Fig. 3). In vitro oxolinic acid blocks the relaxing activity of gyrase (9, 25); therefore, it is unlikely that gyrase is the source of relaxation occurring in vivo in the presence of the drug. Possible alternative sources are topoisomerase III (3), the low (1%) residual activity of topoisomerase I in the *topA* mutant used (strain RS2 [23]), and endonucleolytic cleavage of DNA followed by ligation.

During the course of this work, another example of DNA relaxation in the absence of topoisomerase I was reported (16): relaxation of plasmid DNA occurs in a *topA* deletion mutant carrying a *gyrB*(Ts) mutation after a shift to a nonpermissive temperature.

Thus, there are three cases in which the inhibition of gyrase leads to DNA relaxation when little or no topoisomerase I is present. These observations suggest that topoisomerase I is not the only source of DNA relaxation in wild-type cells, consistent with the presence of other relaxing activities. However, topoisomerase I is clearly an important source of relaxing activity, since both titratable supercoiling and intracellular supercoiling are higher than normal in its absence (17, 18, 20; J. Bliska and N. R. Cozzarelli, submitted for publication). Apparently, some relaxation events require topoisomerase I, whereas others do not. Understanding this dichotomy may help us better understand how DNA supercoiling is regulated.

Two additional comments about our results on DNA relaxation in *topA* mutants deserve note. First, the effects of quinolones on DNA relaxation may vary with the particular

DNA examined and the specific antibiotic tested: Bliska and Cozzarelli (submitted) found that plasmid pJB3.5d DNA does not relax after the addition of norfloxacin to strain RS2 (*topA10*), whereas we found (Fig. 3) that the addition of oxolinic acid to the same strain relaxes chromosomal DNA. Second, our observation that chromosomal DNA relaxation is similar in *topA*⁺ and *topA*⁻ strains treated with coumermycin fails to confirm an expectation arising from the work of Menzel and Gellert (14) showing that the expression of gyrase is induced by DNA relaxation. They found that the rate of induction of DNA gyrase by coumermycin is slower in *topA*⁻ strains than in *topA*⁺ ones, consistent with the expectation that topoisomerase I is responsible for relaxation caused by coumermycin. One possible source for this apparent discrepancy is the different growth conditions used in the two laboratories. In our experiments, cells were grown in a highly enriched minimal medium in which *topA*⁻ and *topA*⁺ strains have similar doubling times: about 45 min for DM800 and its *topA*⁺ derivative SD108. In the experiments of Menzel and Gellert (14), the doubling time of DM800 was 2 to 3 h, about twice as long as that of its *topA*⁺ cognate strain (M. Gellert, personal communication).

Inhibitors of DNA gyrase can, paradoxically, increase the level of bacterial chromosome supercoiling under some conditions. Two examples of this phenomenon have been reported (12): the response of wild-type strains to low levels of coumermycin and the response of a triple topoisomerase mutant (Δ *topA gyrB225 gyrA* [Nal^r]) to oxolinic acid. In the present study, we have extended the characterization of the oxolinic acid-induced increase in supercoiling. The effect

requires partial sensitivity to oxolinic acid and the $\Delta topA$ and $gyrA$ (Nal^r) mutations. We do not know if the $gyrB225$ mutation is also required. Full resistance to oxolinic acid in the triple topoisomerase mutant is conferred by a mutation which maps at or near $gyrB$. This observation suggests that the sensitivity to oxolinic acid seen in strains carrying the $\Delta topA$, $gyrB225$, and $gyrA$ (Nal^r) mutations may be due, in part, to the interaction between the mutant A and B subunits of gyrase and implies that the $gyrB225$ mutation is required. Abnormal subunit interaction cannot be the entire cause of oxolinic acid sensitivity in these strains, however, since a strain carrying only the $gyrA$ (Nal^r) and $gyrB225$ mutations is fully resistant to oxolinic acid (Fig. 4C and Fig. 5B). Perhaps another factor contributing to oxolinic acid sensitivity in the triple mutant is that gyrase expression is reduced in $topA$ deletion mutants (8). Our data do not bear on how oxolinic acid can cause supercoiling to increase. A separate line of investigation (R. J. Franco and K. Drlica, manuscript in preparation) suggests that the increased supercoiling may be due to an increase in gyrase expression.

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