JOURNAL OF BACTERIOLOGY, Dec. 1981, p. 745-752
0021-9193/81/120745-08$02.00/0

Vol. 148, No. 3

Effect of Nalidixic Acid and Novobiocin on pBR322 Genetic Expression in *Escherichia coli* Minicells

M. CARMEN GÓMEZ-EICHELMANN

Departamento de Biología Molecular, Instituto de Investigaciones Biomédicas, UNAM, México 20, D. F., México

Received 15 May 1981/Accepted 12 August 1981

The effects of two deoxyribonucleic acid (DNA) gyrase inhibitors, nalidixic acid and novobiocin, on the gene expression of plasmid pBR322 in *Escherichia coli* minicells were studied. Quantitative estimates of the synthesis of pBR322-coded polypeptides in novobiocin-treated minicells showed that the synthesis of a polypeptide of molecular weight of 34,000 (the tetracycline resistance protein) was reduced to 11 to 20% of control levels, whereas the amount of a polypeptide of 30,500 (the β-lactamase precursor) was increased to as much as 200%. Nalidixic acid affected the synthesis of the tetracycline resistance protein similarly to novobiocin, although to a lesser extent. The effects of nalidixic acid were not observed in a nalidixic-resistant mutant; those induced by novobiocin were only partially suppressed in a novobiocin-resistant mutant. The synthesis of one of the inducible tetracycline-resistant proteins (34,000) coded by plasmid pSC101 was also reduced in nalidixic acid- and novobiocin-treated minicells. These results suggest that the gyrase inhibitors modified the interaction of ribonucleic acid polymerase with some promoters, either by decreasing the supercoiling density of plasmid DNA or by altering the association constant of the gyrase to specific DNA sites.

The *Escherichia coli* enzyme DNA gyrase (topoisomerase II) catalyzes the introduction of negative superhelical turns into closed, circular, double-stranded DNA in an ATP-dependent reaction (7, 13). Gyrase is a tetrameric enzyme composed of two A subunits and two B subunits (14, 28). Subunit A, which is coded for by *gyrA* (*nalA*), determines the sensitivity of the cell to nalidixic (Nal) and oxolinic acids (34); subunit B, coded for by *gyrB* (*cou*), controls the sensitivity to coumermycin A_1 and novobiocin (Nov) (15). The reactions catalyzed by gyrase include an energy-coupled ATP-dependent process that reduces the linking number (8), thus introducing negative supercoils into closed, duplex DNA (13), and a nicking-and-closing reaction resulting in the relaxation of supercoiled DNA in the absence of ATP (12, 34). In *E. coli*, the gyrase catalyzes an additional reaction: the transient cleavage of double-stranded DNA, which leads to the catenation or uncatenation of duplex DNA circles (7, 25). Subunit B mediates the activities requiring ATP, whereas subunit A is associated with reactions that require the concerted breakage and reunion of DNA (33, 34).

DNA gyrase has been shown to be involved in a number of cellular processes, such as supercoiling of the chromosome (11); DNA replication, transcription, and repair (17, 20, 21, 27); λ integrative recombination (26); and general recombination (17). DNA gyrase and, therefore, the degree of DNA supercoiling also have been involved in the selectivity of gene expression. A number of in vivo (30, 32) and in vitro (38) studies in a cell-free transcription-translation system have shown that the gyrase inhibitors nalidixic acid, oxolinic acid, coumermycin A_1, and novobiocin selectively interfere with the transcription of some genes, mainly those of catabolic-sensitive operons. An explanation proposed for these results is that the gyrase inhibitors, by inducing a decrease in the supercoiling density of circular DNA, modify the interaction of RNA polymerase with some promoters (30, 32, 38).

The purpose of this work was to determine and to compare the effects of two different gyrase inhibitors, nalidixic acid and novobiocin, on gene expression of the well-studied small plasmid pBR322 (2, 36) in *E. coli* minicells (1). This plasmid controls the synthesis of those polypeptides which confer the phenotype of resistance to ampicillin (Ap^r) and to tetracycline (Tc^r) on the host cell. These polypeptides have been partially characterized (19), and their transcriptional signals have been mapped on the pBR322 DNA sequence (35, 36). *E. coli* minicells were used since not only do they constitute a techni-

cally simpler system than the in vitro cell-free transcription-translation system, but they also make possible, due to the absence of chromosome-directed protein synthesis, the quantitative analysis of pBR322 gene expression under different experimental conditions.

MATERIALS AND METHODS

Bacterial strains and plasmids. All experiments were performed with derivatives of the minicell-producing *E. coli* K-12 strain P678-54 *thr-1 leu-6 lacY1 minA1 minB2 rpsL135 supE44 thi-1* (1). Plasmid pBR322 (Ap^r Tc^r) (2, 36) was introduced into the minicell-producing strain by transformation, using the calcium chloride method (6). Transformants containing pBR322 were selected for ampicillin resistance on Luria broth plates (23) containing 50 μg of ampicillin per ml. Plasmid pSC101 was obtained from N. Willetts.

Purification of pBR322 DNA. The plasmid DNA used to transform the minicell-producing strain was isolated from cells after amplification with chloramphenicol (170 μg/ml) (3). The lysis of the cells with lysozyme and Triton X-100 and plasmid purification were as described by Bolivar et al. (2).

Isolation of minicells. The minicell-producing strain was grown to early stationary phase in a mineral salt solution (9) supplemented with 0.5% glucose, 0.5% Casamino Acids, essential amino acids (20 μg/ml), and thiamine (0.2 μg/ml). Minicells were purified by the method of Roozen et al. (29), using sucrose step gradients instead of linear gradients. The minicells prepared by this procedure contained less than 10^3 viable cells per 10^9 minicells.

Labeling procedure. The minicells were suspended in 1 ml of labeling medium (24), standardized to an absorbancy of 0.2 at 620 nm, and incubated at 37°C for 15 min. [^{35}S]methionine (25 μCi) was added for protein labeling, and incubation was continued for 30 min. The minicells were centrifuged and suspended in 40 μl of sample buffer (22). The samples were stored at −70°C until used.

Minicell separation into envelope and cytoplasm-periplasm fractions. Labeled minicells in mineral salt solution (9) were treated with EDTA-lysozyme and disrupted by sonication (18). Minicell envelopes were pelleted at 35,000 rpm for 1 h in a Beckman L5-50 centrifuge, using the SW50.1 rotor, and the cytoplasm-periplasm proteins in the supernatant were precipitated with 10% (wt/vol) trichloroacetic acid. The cell envelope and cytoplasm-periplasm fractions were suspended in 40 μl of sample buffer and stored at −70°C as described above.

SDS-PAGE and autoradiography. A 20-μl amount of the thawed minicell samples was heated for 2 min in a boiling water bath and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by the discontinuous buffer system of Laemmli (22). Slab gels (11 by 12 by 0.15 cm) consisted of 4.5% (wt/vol) acrylamide stacking gel (1 cm) and 12.5% (wt/vol) acrylamide separating gel (10 cm). Electrophoresis was carried out at 10 mA for approximately 1 h followed by 20 mA until the bromophenol blue dye front reached the end of the gel (about 4 h). The gels were stained in 0.06% (wt/vol) Coomassie brilliant blue R250 in 30% methanol–10% acetic acid at 37°C for 2 h and were destained in several changes of 10% acetic acid. For autoradiography, Kodak XS-5 X-ray film was exposed, for 1 to 3 days, to the destained gel, which had been dried under vacuum; the film was processed according to standard procedures. The autoradiograms were scanned at 600 nm in a Gilford 250 spectrophotometer coupled with a linear Transport 2410-S, and the areas under the four major peaks were calculated by cutting out and weighing of the peaks.

Chemicals and radiochemicals. Antibiotics and protein molecular weight standards were purchased from Sigma Chemical Co., St. Louis, Mo. Reagents for SDS-PAGE were from Bio-Rad Laboratories, Richmond, Calif. [^{35}S]methionine was obtained from New England Nuclear Corp., Boston, Mass.

RESULTS

The genetic expression of plasmid pBR322, as measured by plasmid-directed polypeptide synthesis in minicells, was studied in the presence of sublethal and lethal concentrations of either nalidixic acid or novobiocin. The polypeptides labeled during the synthesis in minicells containing pBR322, in the presence or absence of the antibiotics, were separated by SDS-PAGE, visualized by autoradiography, and quantified by calculating the areas under the peaks obtained by densitometric scans of the autoradiograms. The densitometric scan of the control experiment (Fig. 1a) showed four major peaks and several discrete minor peaks in the region corresponding to polypeptides of molecular weights of 15,000 to 23,000. The major peaks were assigned as follows: peak 1 (34,000), one of the tetracycline-resistant proteins (Tet protein) coded by a DNA fragment of plasmid pSC101 (5) present in pBR322 (2); peak 2 (30,500), the precursor of β-lactamase (10) coded by a DNA fragment of Tn*3* of plasmid R1-19 (4) present in pBR322 (2); peak 3 (28,500), β-lactamase (10); and peak 4 (25,000), a breakdown product of this enzyme (10). In autoradiograms obtained at shorter exposure times, band 3 was resolved into two discrete bands of approximately 28,000 and 29,000 daltons; autoradiograms obtained at longer exposure times revealed two discrete bands of approximately 30,000 and 26,500 daltons.

Effect of nalidixic acid and novobiocin on pBR322-directed polypeptide synthesis in *E. coli* minicells. Nalidixic acid, even at concentrations as low as 2.5 μg/ml, exerted an inhibitory effect on the synthesis of the above mentioned polypeptides (Fig. 1b and c; Table 1). Nalidixic acid inhibited mainly the synthesis of the polypeptide corresponding to peak 1, which was reduced to 56 to 72%, whereas the amounts of polypeptides 2, 3, and 4 were less affected

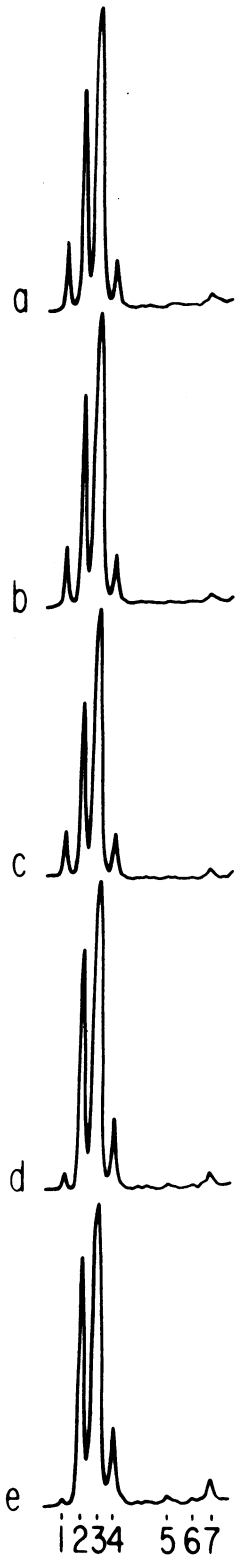


TABLE 1. *Effect of nalidixic acid and novobiocin on pBR322 DNA-directed protein synthesis in E. coli minicells*[a]

Antibiotic	Amt (μg/ml)	Area (%) under labeled protein peak: 1	2	3	4
None		100	100	100	100
Nal	2.5	68–72	91–94	80–87	92–101
Nal	5	60–66	82–94	80–84	80–93
Nal	10	57–63	87–90	76–81	88–92
Nal	25	56–61	90–101	77–82	98–100
Nov	50	12–20	95–108	92–95	92–100
Nov	100	12–17	107–115	100–104	97–101
Nov	200	11–16	121–165	106–112	102–111
Nov	400	11–14	137–200	105–115	110–118
Nov	600	11–15	105–110	95–99	99–105

[a] Labeled extracts from minicells containing pBR322 treated with sublethal (nalidixic acid, 2.5 and 5 μg/ml; novobiocin, 50, 100, 200, and 400 μg/ml) and lethal (nalidixic acid, 10 and 25 μg/ml; novobiocin, 600 μg/ml) concentrations of the gyrase inhibitors were separated by PAGE and autoradiographed as described in the text. The autoradiograms were scanned at 600 nm under identical conditions, and the areas under the four major peaks were calculated. For each peak the values were expressed as a percentage of the corresponding peak of the control experiment (no antibiotic). A total of three individual determinations were done. The range rather than the average is presented. Protein peaks are numbered as in Fig. 1.

(Table 1). In the P678-54 Nal^r derivative strain, the effects of nalidixic acid were not detected, whereas the effect of novobiocin (see below) on the synthesis of polypeptide 1 was approximately the same as was observed in the Nal^s strain (data not shown).

Novobiocin significantly affected the amounts of the polypeptides corresponding to peaks 1 and 2, i.e., the Tet protein and the precursor of β-lactamase, respectively. The former was reduced to 11 to 20%, whereas the latter was increased up to 107 to 200% of the amount synthesized in the minicells incubated without novobiocin (Fig. 1d and e; Table 1). These effects were observable with novobiocin at a concentra-

FIG. 1. *Densitometric scan of the autoradiogram of extracts from [^{35}S]methionine-labeled minicells containing pBR322. Minicells were isolated, preincubated in minimal medium (9) for 15 min, labeled with [^{35}S]methionine for 30 min, and subjected to SDS-PAGE on a 12.5% (wt/vol) separating gel as described in the text. The autoradiogram was scanned at 600 nm. Each track was scanned under identical conditions so that they could be quantitatively compared. (a) No antibiotic present, (b) 2.5 μg of nalidixic acid per ml, (c) 5.0 μg of nalidixic acid per ml, (d) 50 μg of novobiocin per ml, (e) 200 μg of novobiocin per ml. Numbers on the bottom correspond to the major radioactive peaks.*

tion as low as 50 μg/ml. This concentration caused only a slight increase in the generation time of strain P678-54(pBR322) (42 min in L-broth versus 45 min in L-broth containing 50 μg of novobiocin per ml). In addition to its effects on the synthesis of polypeptides 1 and 2, novobiocin also affected the amount of the discrete polypeptide peaks which appeared in the 15,000- to 23,000-dalton region (Fig. 1d and e). Although novobiocin caused an increase in the amount of all polypeptides in this region, the increase was more apparent and reproducible for the 19,500- and 15,000-dalton polypeptides, 5 and 7 (Fig. 1e). The effects of novobiocin were less pronounced in a Nov^r derivative of strain P678-54: novobiocin (200 μg/ml) reduced the amount of polypeptide 1 to 50 to 60% and increased that of polypeptide 2 to 100 to 105% of control levels. Nalidixic acid did not affect significantly the synthesis of polypeptides 1, 2, 3, and 4 on this specific Nov^r strain (data not shown).

Effect of novobiocin on the tetracycline resistance phenotype of *E. coli* cells containing pBR322. Cultures of *E. coli* P678-54 containing pBR322 survived with an efficiency of plating of 98% on Luria broth plates containing 50 μg of tetracycline per ml. Cultures of the same *E. coli* strain grown in the presence of 200 μg of novobiocin per ml showed 40% survival on Luria broth plates containing 50 μg of tetracycline and 200 μg of novobiocin per ml, as compared with the same culture spread onto Luria broth plates containing only 200 μg of novobiocin per ml.

Effect of novobiocin and nalidixic acid on the half-life of the pBR322-coded polypeptides synthesized in minicells. The novobiocin- and nalidixic acid-induced decrease in the amount of the Tet protein and the novobiocin-induced increase in the amount of the β-lactamase-related polypeptides (Table 1) may be explained either as a direct effect of the antibiotic on the synthesis of these polypeptides or as a secondary effect on their degradation. To distinguish between these two possibilities, I carried out a pulse-chase experiment (Fig. 2). In this experiment the cell envelope was separated from the cytoplasm-periplasm, and both fractions, in addition to that of total minicells, were analyzed by SDS-PAGE as described above. The separation of [^{35}S]methionine-labeled minicell samples obtained before and after a 30-min chase in nonradioactive medium, into cells, envelope, and cytoplasm-periplasm fractions allowed a better analysis of the half-life of the pBR322-coded polypeptides synthesized in minicells and of the cellular localization of these polypeptides. The results showed that the amount of the Tet protein (peak 1) remained constant, in relation to the no-chase experiment (Fig. 2a through c), after the 30-min chase performed with or without 200 μg of novobiocin per ml (Fig. 2d through i). It should be noted that the amount of the Tet protein recovered from the envelope fraction (Fig. 2b, e, and h) was greater than that recovered from total minicells (Fig. 2a, d, and g). This was probably due to greater solubilization of the protein from the wall-free envelope fraction than of that from complete minicells.

During the chase, the amount of polypeptide 2 decreased to 68% of control level, and this decrease correlated with an increase in the amount of polypeptide 3 (Fig. 2d through f). A similar correlation was observed when novobiocin was present during the chase (Fig. 3g through i versus a through c) although, in this case, the decrease in polypeptide 2 was lower (62%). However, there was no significant change in the total amount of protein present at the end of the chase, either with or without novobiocin.

The Tet protein (polypeptide 1), the precursor of β-lactamase (polypeptide 2) and, unexpectedly, the breakdown product of this enzyme (polypeptide 4) were localized in the envelope fractions (Fig. 2b, e, and h). In the control experiment (Fig. 2a through c), the β-lactamase was localized mainly in the cytoplasm-periplasm fraction, with approximately 20% present in the envelope fraction.

In the minicell system, the conversion of the β-lactamase precursor into β-lactamase seems to take place via a cell envelope-bound form of the enzyme. As mentioned above, after the chase the decrease in the amount of the β-lactamase precursor (polypeptide 2), which is localized in the envelope fraction, was associated with an increase in the envelope polypeptide of 28,500 daltons (polypeptide 3) (Fig. 2b, e, and h). Polypeptide 4 was localized in the envelope fraction (Fig. 2b, e, and h), although a minor portion of this polypeptide was present in the cytoplasm-periplasm fraction (Fig. 2c, f, and i). Its concentration was reduced to 35% after the 30-min chase, both with and without novobiocin. The decrease in the amount of this polypeptide was associated with an increase in the amount of a soluble polypeptide of approximately 20,500 daltons (Fig. 2c, f, and i). This fact suggests that both polypeptides correspond to breakdown products of β-lactamase. In a pulse-chase experiment similar to that described for novobiocin, nalidixic acid did not cause measurable changes in the relative amounts of pBR322-coded polypeptides after the 30-min chase (data not shown).

Effect of nalidixic acid and novobiocin on

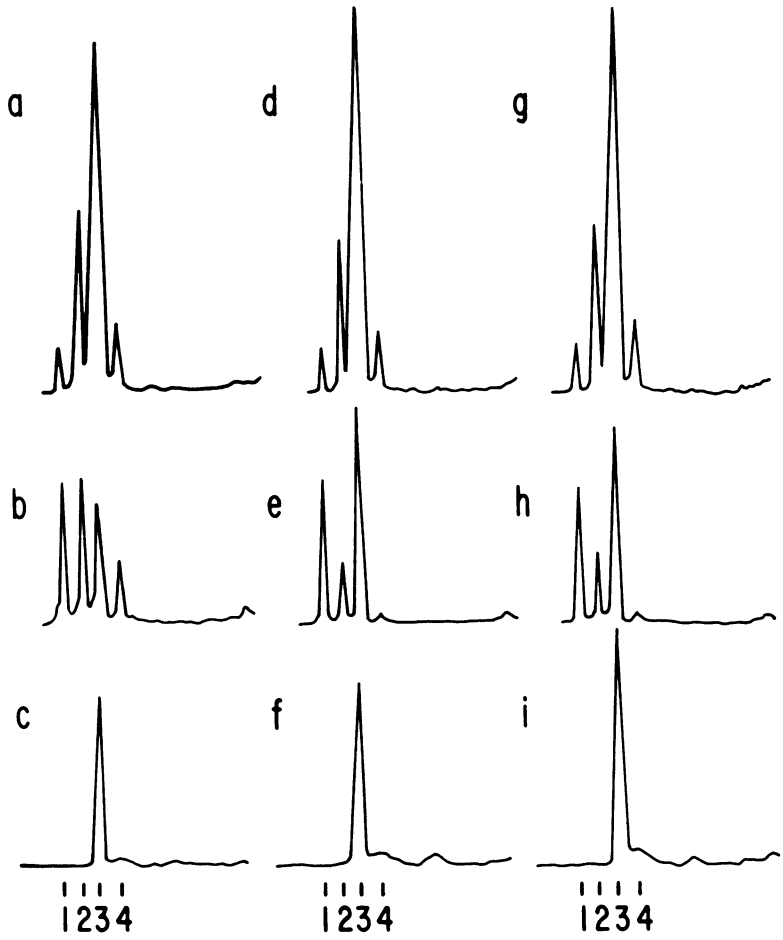
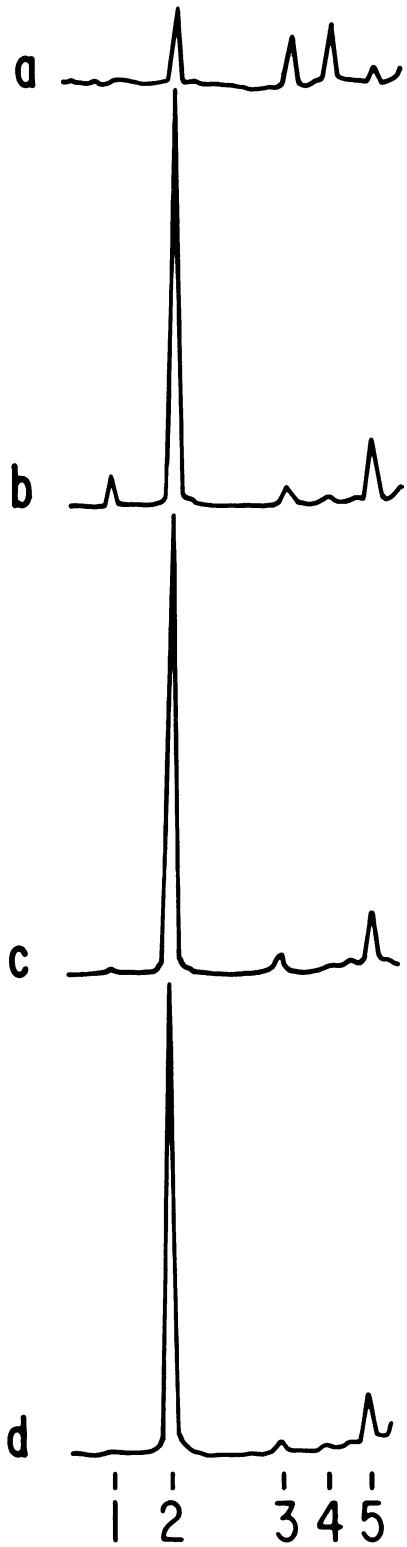


FIG. 2. *Densitometric scan of the autoradiograms of extracts from [^{35}S]methionine-labeled minicells containing pBR322 which were subjected to a pulse-chase procedure. Minicells were isolated, preincubated for 15 min at 37°C, labeled with [^{35}S]methionine for 10 min as described in the text, washed, suspended in minimal medium (11) containing 400 μg of nonradioactive methionine per ml with or without novobiocin, and incubated for 30 min. The labeled minicells were separated into envelope and cytoplasm-periplasm fractions. The polypeptides from these fractions were resolved by SDS-PAGE and visualized by autoradiography as described in the text. (a through c) no chase; (d through f) chase in the absence of novobiocin (g through i) chase in the presence of 200 μg of novobiocin per ml; (a, d, and g) total minicells; (b, e, and h) envelope fractions; (c, f, and i) cytoplasm-periplasm fractions.*

pSC101-directed polypeptide synthesis in *E. coli* minicells. Since the gene that codes for the Tet protein in pBR322 originated in pSC101 (2) and tetracycline resistance is constitutive in pBR322 but inducible in pSC101 (37), it was desirable to investigate whether the nalidixic acid and novobiocin inhibitory effect on its synthesis when directed by pBR322 was also present in minicells containing pSC101. For this purpose, the tetracycline-induced protein synthesis directed by pSC101 in minicells was studied in the presence or absence of the gyrase inhibitors.

Tetracycline induced an increase in the amount of proteins of molecular weights of 34,000 (peak 1), 27,000 (peak 2), and 14,000 (peak 5) and a decrease in that of proteins of molecular weights of approximately 19,000 (peak 3) and 17,000 (peak 4) (Fig. 3a through b). Nalidixic acid and novobiocin inhibited the induction of the 34,000-dalton protein, whereas the induction of the 27,000- and 14,000-dalton proteins, as well as the amounts of the 19,000- and 17,000-dalton proteins, were unaffected (Fig. 3b through d). The amount of the 34,000-dalton protein was reduced when synthesized in the presence of nalidixic acid and novobiocin (Fig. 3c and d). Thus the genetic expression of the Tet protein (34,000 daltons) directed both by pSC101 and



pBR322 was sensitive to the gyrase inhibitors nalidixic acid and novobiocin.

DISCUSSION

It has been shown that nalidixic acid and novobiocin inhibit selectively and reversibly DNA synthesis with little effect on RNA or protein synthesis (16, 31). However, evidence recently was presented showing that gyrase inhibitors decrease the initiation of transcription of some genes (21), thereby decreasing the relative concentration of the protein coded by those genes (30, 32, 38). It has been proposed that the selective gyrase effects on transcription depend on the ease with which the helix is unwound at the promoter site, which is, at least in part, determined by the degree of supercoiling of the DNA (30, 32, 38).

The results presented in this paper demonstrate that the promoter of the gene which codes for the Tet protein (34,000 daltons) in plasmid pBR322 was more sensitive to the gyrase inhibitors nalidixic acid and novobiocin than was the promoter for the gene of the β-lactamase contained in the same plasmid. The novobiocin-induced reduction in the amount of the Tet protein detected in the minicell system (Table 1) was also expressed in the tetracycline phenotype of cells carrying pBR322. The synthesis of the tetracycline-induced 34,000-dalton protein encoded by the plasmid pSC101 was also sensitive to nalidixic acid and novobiocin (Fig. 3). The increase in the amount of the forms of the β-lactamase gene product induced by novobiocin (Table 1) could result from the presence of two partially overlapping promoters at the beginning of the tetracycline region that initiate transcription in the opposite direction (35) or from the fact that the promoter for the *tet* gene is no longer competing for the limited amount of RNA polymerase present in the minicells. On the other hand, the effect of nalidixic acid and novobiocin on the specific Nal^r and Nov^r alleles

FIG. 3. *Densitometric scanning of the autoradiogram of extracts from [^{35}S]methionine-labeled minicells containing pSC101. Minicells containing pSC101 were purified and preincubated for 15 min, with or without 5 μg of tetracycline per ml. [^{35}S]-methionine and either nalidixic acid or novobiocin were added, and incubation was continued for 30 min. Polypeptides were resolved by SDS-PAGE, and radioactive polypeptides were detected by autoradiography as described in the text. (a) Polypeptides synthesized in minimal medium; (b) polypeptides synthesized upon tetracycline induction; (c and d) polypeptides synthesized upon tetracycline induction in the presence of 10 μg of nalidixic acid per ml and 200 μg of novobiocin per ml, respectively.*

used in this work underlines the polyvalent role of the gyrase in gene expression and DNA replication.

The model which is emerging as an explanation of gyrase functions assumes that this enzyme acts at those specific chromosomal sites which determine different domains, thus generating regional differences in processes which are facilitated by the supercoiling of the DNA (7).

Based on this model, two explanations for the results presented in this paper are offered. The first assumes that the requirement that DNA be supercoiled to facilitate recognition by RNA polymerase and therefore transcription is higher for the promoter of the gene that codes for the 34,000-dalton protein than it is for the promoter of the β-lactamase gene. As was mentioned above, the requirement for gyrase in the transcription of some genes has been related to supercoiling, which facilitates unwinding of the helix at the promoter site. Thus, it can be predicted that promoters localized in DNA regions with a low percentage of adenine·thymine will show a higher requirement for gyrase. In this respect, it should be mentioned that the percentages of adenine·thymine in the 100 base pairs preceding the putative ATG starting codons for the Tet protein and β-lactamase gene (36) are 44 and 71%, respectively.

The alternative explanation is based on the report that oxolinic acid induces a tight gyrase binding at specific DNA sites (12, 14, 34). This modified gyrase-DNA interaction could lead to differences in the transcription of genes, depending upon the localization of the bound gyrase relative to the gene promoters. A gyrase bound at or close to a promoter could prevent recognition by RNA polymerase of that promoter. If this explanation is correct, one would expect nalidixic acid and novobiocin to induce a tight gyrase binding at or close to the Tet promoter, but not at or close to the β-lactamase promoter. This alternative interpretation could also explain the sensitivity of transcription of linear DNA to gyrase inhibitors (38).

ACKNOWLEDGMENTS

I thank Estela García for preparing the bacterial media, R. Ruiz and G. Alvarez for technical assistance, and Rosa Ma. Cuevas for typing the manuscript. I also thank V. Yakoleff Greenhouse for excellent editorial assistance.

LITERATURE CITED

1. **Adler, H. I., W. D. Fisher, A. Cohen, and A. A. Hardigree.** 1966. Miniature *Escherichia coli* cells deficient in DNA. Proc. Natl. Acad. Sci. U.S.A. **57**:321–326.
2. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
3. **Clewell, D. B.** 1972. Nature of ColE1 plasmid replication in *Escherichia coli* in the presence of chloramphenicol. J. Bacteriol. **110**:667–676.
4. **Cohen, S. N.** 1977. Special sequences in the structure of cointegrate drug resistance plasmids related to F, p. 672–673. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. **Cohen, S. N., and A. C. Y. Chang.** 1973. Recircularization and autonomous replication of a sheared R-factor DNA segment in *Escherichia coli* transformants. Proc. Natl. Acad. Sci. U.S.A. **70**:1293–1297.
6. **Cohen, S. N., A. C. Y. Chang, and L. Hsu.** 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. Proc. Natl. Acad. Sci. U.S.A. **69**:2110–2114.
7. **Cozzarelli, N. R.** 1980. DNA gyrase and the supercoiling of DNA. Science **207**:953–960.
8. **Crick, F. H. C.** 1976. Linking numbers and nucleosomes. Proc. Natl. Acad. Sci. U.S.A. **73**:2639–2643.
9. **Curtiss, R., III.** 1965. Chromosomal aberrations associated with mutations to bacteriophage resistance in *Escherichia coli*. J. Bacteriol. **89**:28–40.
10. **Dougan, G., M. Saul, A. Twigg, R. Gill, and D. Sherratt.** 1979. Polypeptides expressed in *Escherichia coli* K-12 minicells by transposition elements Tn*1* and Tn*3*. J. Bacteriol. **138**:48–54.
11. **Drlica, K., and M. Snyder.** 1978. Superhelical *Escherichia coli* DNA: relaxation by coumermycin. J. Mol. Biol. **120**:145–154.
12. **Gellert, M., K. Mizuuchi, M. H. O'Dea, T. Itoh, and J. Tomizawa.** 1977. Nalidixic acid resistance: a second genetic character involved in DNA gyrase activity. Proc. Natl. Acad. Sci. U.S.A. **74**:4772–4776.
13. **Gellert, M., K. Mizuuchi, M. H. O'Dea, and H. A. Nash.** 1976. DNA gyrase: an enzyme that introduces superhelical turns into DNA. Proc. Natl. Acad. Sci. U.S.A. **73**:3872–3876.
14. **Gellert, M., K. Mizuuchi, M. H. O'Dea, H. Ohmori, and J. Tomizawa.** 1978. DNA gyrase and DNA supercoiling. Cold Spring Harbor Symp. Quant. Biol. **43**:35–40.
15. **Gellert, M., M. H. O'Dea, T. Itoh, and J. Tomizawa.** 1976. Novobiocin and coumermycin inhibit DNA supercoiling catalyzed by DNA gyrase. Proc. Natl. Acad. Sci. U.S.A. **73**:4474–4478.
16. **Goss, W. A., W. H. Dietz, and T. M. Cook.** 1965. Mechanism of action of nalidixic acid on *Escherichia coli*. II. Inhibition of deoxyribonucleic acid synthesis. J. Bacteriol. **89**:1068–1074.
17. **Hays, J., and S. Boehmer.** 1978. Antagonists of gyrase inhibit repair and recombination of UV-irradiated phage λ. Proc. Natl. Acad. Sci. U.S.A. **75**:4125–4129.
18. **Kennedy, N., L. Beutin, M. Achtman, R. Skurray, U. Rahmsdorf, and P. Herrlich.** 1977. Conjugation proteins encoded by the sex factor. Nature (London) **270**: 580–585.
19. **Kopylova-Sviridova, T. N., V. V. Soukovatitsin, and I. Fodor.** 1979. Synthesis of proteins coded by plasmid vectors of pCV series (Ap^r, Tc^r) and their recombinant derivatives (pDm) in *E. coli* minicells. Gene **7**:121–139.
20. **Kreuzer, K. N., and N. R. Cozzarelli.** 1979. *Escherichia coli* mutants thermosensitive for deoxyribonucleic acid gyrase subunit A: effects on deoxyribonucleic acid replication, transcription, and bacteriophage growth. J. Bacteriol. **140**:424–435.
21. **Kubo, M., Y. Kano, H. Nakamura, A. Nagata, and F. Imamoto.** 1979. In vivo enhancement of general and specific transcription in *Escherichia coli* by DNA gyrase activity. Gene **7**:153–171.
22. **Laemmli, U. K.** 1970. Cleavage of structural proteins

during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
23. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190–206.
24. **Meagher, R. B., R. C. Tait, M. Betlach, and H. W. Boyer.** 1977. Protein expression in *E. coli* minicells by recombinant plasmids. Cell **10**:521–536.
25. **Mizuuchi, K., L. M. Fisher, M. H. O'Dea, and M. Gellert.** 1980. DNA gyrase action involves the introduction of transient double-strand breaks into DNA. Proc. Natl. Acad. Sci. U.S.A. **77**:1847–1851.
26. **Mizuuchi, K., M. Gellert, and H. A. Nash.** 1978. Involvement of supertwisted DNA in integrative recombination of bacteriophage lambda. J. Mol. Biol. **121**: 375–392.
27. **Orr, E., N. F. Fairweather, I. B. Holland, and R. H. Pritchard.** 1979. Isolation and characterization of a strain carrying a conditional lethal mutation in the *cou* gene of *Escherichia coli* K-12. Mol. Gen. Genet. **177**: 103–112.
28. **Peebles, C. L., N. P. Higgins, K. N. Kreuzer, A. Morrison, P. O. Brown, A. Sugino, and N. R. Cozzarelli.** 1978. Structure and activities of *Escherichia coli* DNA gyrase. Cold Spring Harbor Symp. Quant. Biol. **43**:41–52.
29. **Roozen, K. J., R. G. Fenwick, Jr., and R. Curtiss III.** 1971. Synthesis of ribonucleic acid and protein in plasmid-containing minicells of *Escherichia coli* K-12. J. Bacteriol. **107**:21–33.
30. **Sanzey, B.** 1979. Modulation of gene expression by drug affecting deoxyribonucleic acid gyrase. J. Bacteriol. **138**:40–47.
31. **Smith, D. H., and B. D. Davis.** 1967. Mode of action of novobiocin in *Escherichia coli*. J. Bacteriol. **93**:71–79.
32. **Smith, C. L., M. Kubo, and F. Imamoto.** 1978. Promoter-specific inhibition of transcription by antibiotics which act on DNA gyrase. Nature (London) **275**:420–423.
33. **Sugino, A., N. P. Higgins, P. O. Brown, C. L. Peebles, and N. R. Cozzarelli.** 1978. Energy coupling in DNA gyrase and the mechanism of action of novobiocin. Proc. Natl. Acad. Sci. U.S.A. **75**:4838–4842.
34. **Sugino, A., C. L. Peebles, K. N. Kreuzer, and N. R. Cozzarelli.** 1977. Mechanism of action of nalidixic acid: purification of *Escherichia coli nal*A gene product and its relationship to DNA gyrase and a novel nicking-closing enzyme. Proc. Natl. Acad. Sci. U.S.A. **74**:4767–4771.
35. **Stüber, D., and H. Bujard.** 1981. Organization of transcriptional signals in plasmids pBR322 and pACYC184. Proc. Natl. Acad. Sci. U.S.A. **78**:167–171.
36. **Sutcliffe, J. G.** 1978. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Cold Spring Harbor Symp. Quant. Biol. **43**:77–90.
37. **Tait, R. C., and H. W. Boyer.** 1978. On the nature of tetracycline resistance controlled by the plasmid pSC101. Cell **13**:73–81.
38. **Yang, H. L., K. Heller, M. Gellert, and G. Zubay.** 1979. Differential sensitivity of gene expression *in vitro* to inhibitors of DNA gyrase. Proc. Natl. Acad. Sci. U.S.A. **76**:3304–3308.