Effect of DNA Gyrase Inhibitors and Urea on the Expression of *cysB*, the Regulatory Gene of the Cysteine Regulon

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cysB, the regulatory gene of the cysteine regulon, is autoregulated. Inhibitors of both gyrase subunits, nalidixic acid and novobiocin, affect the expression of *cysB*, as monitored by β -galactosidase activity in *cysB* : *lac* fusion strains. In *gyrA* mutants that are resistant to nalidixic acid, this drug does not affect *cysB* expression. The amount of mRNA transcribed from the *cysB* promoter isolated from cultures grown in the presence of gyrase inhibitors was significantly lower than that from the control culture without inhibitors. Urea also decreased *cysB* expression. These results suggest that DNA topology could play a role in *cysB* expression.

INTRODUCTION

The degree of supercoiling of DNA appears to be an important factor in control of gene expression (Smith, 1981). The DNA of bacteria, bacteriophages and plasmids is generally topologically constrained in a negatively supercoiled conformation. DNA supercoiling *in vivo* is a result of the balance between topoisomerase I and DNA gyrase, a type of topoisomerase II. DNA gyrase introduces negative superhelical turns into DNA and is composed of two subunits, A and B, encoded by *gyrA* and *gyrB*. Mutations that confer resistance to nalidixic acid or oxolinic acid map in *gyrA*, whereas mutations that result in coumermycin or novobiocin resistance map in *gyrB*.

Superhelical DNA is required for replication, plasmid maintenance and recombination of cellular DNA (Drlica, 1984). *In vitro* studies have shown that the superhelical density influences initiation of transcription (Botchan *et al.*, 1973) and that changes in superhelicity affect different promoters differently (Botchan, 1976). In addition, *in vivo* and *in vitro* studies have shown that some genes are sensitive to gyrase inhibitors. It has been demonstrated that a reduction of supercoiling is strongly correlated with a change of gene expression (Shuman & Schwartz, 1975; Smith *et al.*, 1978; Kubo *et al.*, 1979; DiNardo *et al.*, 1982). Several studies have suggested that promoters under cAMP receptor protein regulation are particularly sensitive to gyrase inhibitors (Sanzey, 1979). However, work on gyrase inhibitors is complicated by these drugs having pleiotropic effects and causing severe growth inhibition. This makes urea particularly useful since it specifically affects gene expression without interfering with overall cellular metabolism (Sanzey & Ullman, 1980).

cysB is the regulatory gene of the cysteine regulon. Although considerable information is available concerning the regulation of the cysteine biosynthesis genes, little is known about the mechanism of *cysB* expression. Jagura-Burdzy & Hulanicka (1981) have reported that *cysB* is autoregulated, but no other mechanisms of *cysB* regulation are known. It was thus of interest to study the relationship between DNA topology and *cysB* expression.

METHODS

Bacterial strains. All strains used were derivatives of *Escherichia coli* K12. Their characteristics are described in Table 1.

Table 1. *E. coli* strains and plasmid used

Strain	Genotype*	Reference or source
MC4100	<i>araD139 Δ(lac)U169 strA relA thi</i>	M. J. Casadaban, Univ. of Chicago, USA
EC1250	<i>araD139 Δ(lac)U169 strA relA thi trp</i>	Jagura-Burdzy & Hulanicka (1981)
EC1173	<i>araD139 Δ(lac)U169 strA relA thi trp cysB88::Mu d(Ap^r lac)</i>	This work
EC1135	<i>araD139 Δ(lac)U169 strA relA thi trp cysE86::Mu d(Ap^r lac)</i>	Bielińska <i>et al.</i> (1984)
EC1230	<i>araD139 Δ(lac-pro) thi trp cysB82::lac</i>	Jagura-Burdzy & Hulanicka (1981)
EC1841	EC1173 <i>gyrA1</i>	This work
EC1842	EC1173 <i>gyrA2</i>	This work
E101	<i>dnaF_{ts} thr-1 thi-1 thyA6 deoC1 supE44 lacY tonA2 str</i>	Univ. of Gdańsk, Poland
Plasmid		
pLG400	Contains a fragment of <i>lacZ</i> (Zabeau & Stanley, 1982)	Institute of Genetics, Univ. of Warsaw, Poland

* The genetic nomenclature is from Bachmann *et al.* (1976).

Media and growth conditions. Sulphate-free medium (SF) was the minimal medium C, in which the sulphur source was 1 mM-L-cystine, 1 mM-L-djenkolate or 1 mM-glutathione. Glucose was added to 0.5%. All solid media contained 1.5% (w/v) agar. Bacterial cultures were incubated with aeration at 37 °C. Antibiotics were added when cultures reached OD₆₅₀ = 0.2–0.3.

Enzyme assays. β-Galactosidase was assayed in toluenized cells with *o*-nitrophenyl-β-D-galactopyranoside as substrate (Miller, 1972). *O*-Acetylserine sulphydrylase was determined as described previously (Kredich, 1971). Sulphite reductase was assayed by the method of deVito & Dreyfuss (1964). Protein was determined by the biuret method using bovine serum albumin as a standard. DNA content in cells was determined by the Burton method with the modification of Giles & Meyers (1965).

Isolation of *gyrA* mutants. Mutations were induced by adding 0.1 ml 0.1% *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine to 5 ml culture and shaking at 37 °C for 30 min; 0.1 ml mutagenized culture was added to 10 ml LB and grown overnight. Spontaneous and *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine-induced nalidixic acid resistant mutations of the fusion strains were isolated by plating about 10⁸ cells on the complete or minimal solid medium containing 20 µg nalidixic acid ml⁻¹. The corresponding *gyrA* mutations were identified by cotransduction with *dnaF* by phage P1vir transduction.

Isolation of RNA and plasmid DNA. Total bacterial RNA was isolated by the phenol method (Ikemura & Dahlberg, 1973). Plasmid DNA was isolated by the method of Holmes & Quigley (1982).

Preparation of ³²P-labelled plasmid DNA probe. Plasmid pLG400 DNA was labelled by nick translation (Mackey *et al.*, 1977) with [³²P]ATP or [³²P]CTP (410 Ci mmol⁻¹, 15 TBq mmol⁻¹) (Amersham).

Electrophoresis, transfer and hybridization. Samples of total bacterial RNA were separated by electrophoresis through a 1.7% agarose gel with 2.2 M-formaldehyde. RNA was transferred from agarose to nitrocellulose paper by using 3M-NaCl/0.3 M-trisodium citrate (20× SSC) as described for DNA transfer by Southern (1975). Hybridization was done using the method of Wahl *et al.*, (1979).

RESULTS

*Effect of gyrase inhibitors on the expression of *cysB**

As the product of *cysB* has no enzyme activity, we used fusion strains where the *lac* operon was fused to the regulatory region of *cysB* to study its expression. In such strains, β-galactosidase synthesis is under the control of the regulatory elements of the *cysB* system. The integration of the phage Mu d1(Ap^r *lac*) into *cysB* was checked by P1 transduction and transformation with plasmids containing *cysB*.

To study the effect of gyrase inhibitors on *cysB* expression, overnight inocula of fusion strains were diluted and grown at 37 °C with shaking. Different concentrations of inhibitors of the A and B subunits of gyrase, nalidixic acid and novobiocin respectively, were added when the OD₆₅₀ reached 0.2–0.3. After two to three generations, samples were taken and β-galactosidase assayed (Miller, 1972). Both drugs inhibited expression of *cysB*, as monitored by β-galactosidase activity, and the inhibition increased with increasing drug concentration (Table 2). Nalidixic acid also inhibited DNA replication. Even a low concentration (5 µg ml⁻¹) stopped DNA synthesis, as measured by the Burton method (data not shown). (Nalidixic acid concentrations higher than 40 µg ml⁻¹ could not be tested because growth was affected.)

To test whether the observed decrease of *cysB* expression was a result of inhibition of DNA

Table 2. *Effect of nalidixic acid and novobiocin on β -galactosidase activity in *cys::lac* fusion strains*

β -Galactosidase activities of control cultures without inhibitors were taken as 100%; these values were 730, 460 and 230 Miller units (Miller, 1972) for EC1173, EC1230 and EC1135 respectively. The results are means of three to five experiments; the deviations from these means were less than 7%.

Strain	Pertinent genotype	Nalidixic acid ($\mu\text{g ml}^{-1}$)	β -Galactosidase activity (% of control)	Novobiocin ($\mu\text{g ml}^{-1}$)	β -Galactosidase activity (% of control)
EC1173	<i>cysB88::lac</i>	0	100	0	100
		10	17	20	64
		20	8	40	32
		40	7	80	25
EC1230	<i>cysB82::lac</i>	0	100	0	100
		20	93	80	95
EC1135	<i>cysE86::lac</i>	0	100	0	100
		20	93	80	100

Table 3. *Effect of nalidixic acid on β -galactosidase activity in *cysB::lac gyrA* mutants*

β -Galactosidase activities of control cultures without inhibitors were taken as 100%; these values were 730, 680 and 720 Miller units (Miller, 1972) for EC1173, EC1841 and EC1842 respectively. The results are means of three experiments; the deviations from these means were less than 10%.

Strain	Pertinent genotype	Nalidixic acid ($\mu\text{g ml}^{-1}$)	β -Galactosidase activity (% of control)
EC1173	<i>cysB88::lac gyrA⁺ Nal^s</i>	0	100
		40	10
EC1841	<i>cysB88::lac gyrA1 Nal^r</i>	0	100
		40	95
EC1842	<i>cysB88::lac gyrA2 Nal^r</i>	0	100
		40	96

synthesis, two fusion strains were used as controls: EC1230 (*cysB::lac*) and EC1135 (*cysE86::lac*). Previously it had been found that *cysB* expression was not autoregulated in strain EC1230 (Jagura-Burdzy & Hulanicka, 1981). Probably some mutation had arisen in the regulatory region of *cysB* in the course of isolating the fusion. The second control strain, EC1135, carries the fusion in *cysE*, which is not affected by gyrase inhibitors (Ostrowski & Hulanicka 1981). The lack of effect of both drugs on the expression of fused genes in control strains (Table 2) indicated the specific involvement of gyrase in expression of *cysB*. Since the inhibitors of both subunits of gyrase affect *cysB* expression, it is likely that the whole gyrase complex is involved in *cysB* expression. Similar results were observed with all other *cysB* fusion strains studied.

To test if the effect of nalidixic acid concerns the *gyrA* product, the action of this drug on a resistant strain was studied. In fusion strains, nalidixic acid resistant mutations were isolated and their location in *gyrA* was confirmed by P1 transduction. In all *gyrA* nalidixic acid resistant strains, even at high concentrations (40 $\mu\text{g ml}^{-1}$), the drug had no effect on the level of β -galactosidase (Table 3). This indicates that the effect of nalidixic acid is specific and that the *gyrA* product is involved in the expression of *cysB*.

Effects of urea on cysB expression

Gyrase inhibitors preferentially inhibit the expression of some genes (Wei-jue *et al.* 1982). However, these drugs severely inhibit growth. At some concentrations urea inhibits the expression of catabolite sensitive genes without influencing bacterial growth (Sanzey & Ullman, 1980). It was of interest to test if urea, which inhibits the expression of cysteine structural genes (Bielinska *et al.* 1984), affects the expression of *cysB*. Thus, the effect of urea on the β -galactosidase activity in the *cysB::lac* fusion strains was examined (Table 4). Urea inhibited *cysB* expression, and the inhibition increased with increasing urea concentration. Higher concentrations (above 0.6 M) were not examined because bacterial growth was affected. As in the previous experiments the control strains EC1230 and EC1135 were used. The inhibition of

Table 4. *Effect of urea on β -galactosidase activity in *cys::lac* fusion strains*

β -Galactosidase activities of control cultures without inhibitors were taken as 100%; these values were 730, 460 and 230 Miller units (Miller, 1972) for EC1173, EC1230 and EC1135 respectively. The results are means of three experiments; the deviations from these means were less than 10%.

Strain	Pertinent genotype	Urea (M)	β -Galactosidase activity (% of control)
EC1173	<i>cysB88::lac</i>	0.0	100
		0.2	70
		0.4	30
		0.6	22
EC1230	<i>cysB82::lac</i>	0.0	100
		0.4	80
EC1135	<i>cysE86::lac</i>	0.0	100
		0.4	110

cysB expression was specific since urea had little effect on the expression of *lacZ* in both control strains. To check if urea interfered with DNA replication, the DNA content of cells grown on different concentrations of this compound was assayed by the Burton method; even at 0.4 M-urea there was no interference with DNA synthesis (not shown). This result, and the lack of effect of urea on gene expression in the control strains, indicated that the decrease in β -galactosidase activity observed in the *cysB::lac* fusion strains was caused by specific interference with *cysB* expression by urea.

*Effect of gyrase inhibitors and urea on the transcription of *cysB**

To show that gyrase inhibitors affect *cysB* expression at the transcriptional level, the amount of the transcript of *lac* mRNA directed from the *cysB* promoter was measured by hybridization using [32 P]DNA of plasmid pLG400 as the probe. This plasmid contains a fragment of the *lac* operon without its own regulatory sequence (Zabeau & Stanley, 1982). Total RNA was isolated from fusion strain EC1173 and also from EC1230 as the control, since *cysB* expression in the latter strain is not autoregulated and is insensitive to gyrase inhibitors and urea. Both strains were grown in the presence and absence of nalidixic acid (20 μ g ml $^{-1}$) and urea (0.4 M). Isolated RNA was separated on an agarose-formaldehyde gel, transferred to nitrocellulose paper and hybridized with the probe.

Distinct bands of *lac* mRNA, visualized by hybridization and autoradiography, occurred in RNA isolated from cultures without inhibitors (Fig. 1, lanes 1 and 4), whereas the presence of nalidixic acid or urea significantly decreased the amount of mRNA directed from the *cysB* promoter in strain EC1173 (Fig. 1, lanes 2 and 3). Similarly, as in the experiments in which β -galactosidase was assayed, these compounds had no effect on the expression of *cysB* in fusion strain EC1230, although the bands (lanes 5 and 6) were slightly less intense than the control band (lane 4). Thus, a comparison of the RNA bands of EC1173 and EC1230 strains grown in the presence of inhibitors shows a significant difference in their intensity.

DISCUSSION

Recently there have been a number of indications that negative supercoiling of DNA stimulates the expression of many genes. Superhelical tension facilitates initiation of transcription and individual genes respond to changes in the degree of supercoiling. Yang *et al.* (1979) have studied the effect of gyrase inhibitors on the coupled transcription-translation system in a DNA-directed cell-free system. They found that the expression of different genes is reduced to different extents by inhibition of DNA gyrase activity. Smith *et al.* (1978) reported that transcription of the *trp* promoter carried on phage ϕ 80 is insensitive to nalidixic acid. In contrast, this drug blocks transcription from the P_L promoter of the same phage. Some other promoters, e.g. *thr* and early T7, are unaffected by gyrase inhibitors, while *malP-malQ*, *malK-lamB*, *lac*, *gal*, *tna* and T7 late promoters are sensitive to these drugs (Schuman & Schwartz, 1975; DeWynagaert & Hinkle, 1979; Drlica, 1984). The antibiotic coumermycin and temperature sensitive mutations affecting gyrase activity decrease RNA synthesis *in vivo* (Drlica, 1984).

Fig. 1. Hybridization of RNA isolated from strains EC1173 (lanes 1, 2 and 3) and EC1230 (lanes 4, 5 and 6) with DNA of plasmid pLG400. The bacteria were grown in the presence of: no inhibitors (lanes 1 and 4); 20 μ g nalidixic acid ml^{-1} (lanes 2 and 5); 0.4 M-urea (lanes 3 and 6).

These treatments probably affect transcription by relaxing DNA superhelical tension. Significantly less is known about the mode of action of urea. Sanzey & Ullman (1980) reported that urea interferes mainly with the initiation of transcription. Other authors (Levine *et al.*, 1963) have shown that urea at a concentration similar to that used in our studies caused changes in the thermodenaturation profile of DNA. If the effect of urea *in vivo* is similar it is conceivable that urea could act by 'freezing' promoters and thus inhibiting the formation of an active complex. Promoters requiring additional activators for the binding of RNA polymerase might be more sensitive to urea.

In this paper we have shown that gyrase inhibitors and urea, the latter of which does not interfere with the overall cellular metabolism, decrease expression of *cysB*, the regulatory gene of the cysteine regulon. Nalidixic acid and novobiocin also inhibit DNA replication. However, the decrease of *cysB* expression is not caused by gene dosage effect, since it is not observed in the control strains EC1230 (*cysB82::lac*) and EC1135 (*cysE86::lac*). Previously it had been shown that *cysE* expression is not affected by gyrase inhibitors (Ostrowski & Hulanicka, 1981). The fusion *cysB82::lac* is not autoregulated (Jagura-Burdzy & Hulanicka, 1981), and fine genetic mapping by P1 transduction showed that the fusion is within the regulatory region of *cysB* (Jagura-Burdzy, 1980). The fusion *cysB82::lac* was constructed by a two step technique (Casadaban, 1976); probably during isolation of this fusion an additional mutation arose in the promoter region which rendered *cysB* insensitive to autoregulation and independent on supercoiling for transcription. A similar case was reported for the lactose operon; in the *lacUV5* mutant a change of two base pairs in the promoter region rendered this strain insensitive to catabolite repression and gyrase inhibitors (Wei-jue *et al.*, 1982).

Urea, like gyrase inhibitors, decreased *cysB* expression and its effect was specific since it did not affect expression in control fusion strains (EC1135, EC1230) (Table 4 and Fig. 1). The decrease of *cysB* expression by inhibitors of DNA gyrase and by urea suggests that DNA topology might play a role in the regulation of expression of this gene.

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