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Increased expression of biodegradative threonine dehydratase of *Escherichia coli* by DNA gyrase inhibitors

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1. SUMMARY

The synthesis of inducible biodegradative threonine dehydratase of *Escherichia coli* increased several-fold in the presence of the DNA gyrase inhibitors, nalidixic acid and coumermycin. Temperature-sensitive *gyrB* mutants expressed higher levels of dehydratase as compared to an isogenic *gyrB*$^+$ strain. Immunoblotting experiments showed increased synthesis of the dehydratase protein in the presence of gyrase inhibitors; addition of rifampicin and chloramphenicol to cells actively synthesizing enzyme preventing new enzyme production. Increased expression of dehydratase by gyrase inhibitors was accompanied by relaxation of supercoiled DNA.

2. INTRODUCTION

In topological terms, chromosomal DNA in bacteria appears to exist in the negative supercoiled state. The extent of DNA supercoiling is dependent, in part, on the ratio of the activities of two enzymes, DNA gyrase, which introduces negative supercoils, and topoisomerase I, which relaxes the supercoiled form [11]. Certain antibiotics and drugs, notably nalidixic acid, coumermycin and novobiocin are known to be potent inhibitors of DNA gyrase [1,2]. Accumulating evidence indicates that in *Escherichia coli* gyrase inhibitors influence transcription of a number of anaerobically expressed genes most likely by altering the state of DNA supercoiling [3–5]. Recently, the *tdcABC* operon of *E. coli* has been cloned, sequenced and expressed in this laboratory [6,7], and the gene products implicated in anaerobic threonine metabolism. Experiments presented in this report reveal that gyrase inhibitors stimulated the expression of biodegradative threonine dehydratase (EC 4.2.1.16), the product of the *tdcB* gene, which catalyzes the dehydration of L-threonine and L-serine to ammonia and the corresponding 2-oxo acids.

3. MATERIALS AND METHODS

3.1. Bacterial strains

E. coli strains K37 (*gyrB*$^+$), K1870 (*gyrB-him-230*(Ts)) and K1871 (*gyrB-him-231*(Ts)) [8] were

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obtained from D.I. Friedman. Strains KL226, JA194, and MC4100 were from this laboratory [6,7].

3.2. Enzyme assay

Cells previously grown aerobically at 37°C in tryptone yeast extract medium (TYE) [9] were washed and resuspended in fresh TYE, and then incubated anaerobically (in still culture) at 30°C for enzyme induction [9]. Threonine dehydratase activity was assayed with toluene-treated cells [9] and the enzyme specific activity is expressed as nmol of 2-oxobutyrate produced per min/10^9 cells.

3.3. DNA preparation and gel electrophoresis

Plasmid DNA was isolated by the method of Agellon and Chen [10]. The extent of DNA supercoiling was assessed by electrophoresis through 1% agarose-chloroquine gels in TBE buffer [5] for 17 h at room temperature at 2.5 V/cm. Both the gel and the buffer contained 1 μg of chloroquine per ml. Under these conditions, supercoiled DNA migrated farther into the gel whereas the more relaxed topoisomers migrated more slowly [5].

3.4. Immunoblotting procedure

Cells (in 3 ml TYE) incubated anaerobically for 5 h at 30°C with and without gyrase inhibitors were harvested, washed twice and then suspended in 0.15 ml of the SDS-sample buffer of Laemmli [11]. Portions of cell lysate (22 μg protein each) were electrophoresed through an 11% SDS-polyacrylamide gel [11], and then electrophoretically transferred to a nitrocellulose membrane according to Towbin et al. [12]. The membrane was probed with affinity-purified threonine dehydratase antibodies [9], and the bound anti-TdcB andtibodies were detected by peroxidase staining.

4. RESULTS

4.1. Effect of gyrase inhibitors on enzyme expression

When incubated anaerobically in TYE the specific activity of threonine dehydratase in strain K37 increased maximally to 5- and 4-fold, respectively, in the presence of the gyrase inhibitors, nalidixic acid (NA) and coumermycin (CM) (Fig. 1). Several other strains of *E. coli* including MC4100, JA194 and KL226 also showed increased enzyme level (1.5-fold or higher) when incubated with NA or CM in TYE. Control experiments indicated that low concentrations of gyrase inhibitors had no effect on cell growth, and the inhibitors did not influence the activity of the purified enzyme (data not shown).

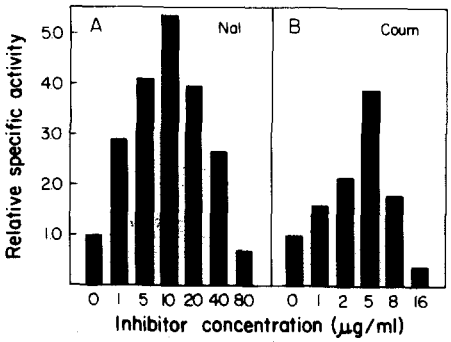

Fig. 1. Stimulation of denhydratase activity by gyrase inhibitors. Cells were incubated anaerobically for 6 h in TYE with various concentrations of nalidixic acid (Nal) and coumermycin (Coum) as indicated.

4.2. De novo synthesis of dehydratase by gyrase inhibitors

Evidence that the high levels of enzyme activity in the presence of gyrase inhibitors was due to de novo protein synthesis was revealed by the following experiments. The addition of 50 μg/ml of rifampicin to cells along with NA (or CM) prevented new enzyme production (Table 1). Similar results were observed when chloramphenicol was added to block protein synthesis (data not shown).

Table 1

Rifampicin effect on the nalidixic acid-stimulated dehydratase synthesis

Additions [a]	Enzyme specific activity [b]
None	120 (1.0)
+ rifampicin (50 μg/ml)	70 (0.58)
+ nalidixic acid (15 μg/ml)	700 (5.9)
+ rifampicin + nalidixic acid	140 (1.2)

[a] Nalidixic acid was added at the beginning of anaerobic incubation; rifampicin was added at 4 h.

[b] For enzyme assay cells were harvested after 6 h of anaerobic incubation in TYE.

Furthermore, immunoblotting with antidehydratase antibodies showed increased synthesis of the dehydratase protein in the presence of the gyrase inhibitors (Fig. 2).

4.3. Threonine dehydratase levels in gyrB *mutants*

If the gyrase inhibitors increased dehydratase synthesis by decreasing the in vivo activity of DNA gyrase, gyrase-deficient mutants should exhibit higher basal levels of dehydratase activity. Upon anaerobic incubation at 31°C in TYE the specific activities of threonine dehydratase in strains K1870 and K1871 harboring temperature-sensitive gyrase B mutations [8] were, respectively, 200 and 210, as compared to a value of 94 for the isogenic parent K37. Friedman et al. [8] have shown that the *gyrB* mutants exhibited approximately 10% of the wild-type GyrB activity seen in K37. Thus, a deficiency of gyrase activity in vivo showed increased expression of dehydratase by about 2-fold.

4.4. Relaxation of plasmid DNA supercoiling in coumermycin-treated cells

Dorman et al. [5] reported that plasmid DNA isolated from anaerobically grown *E. coli* was negatively supercoiled; when anaerobically grown

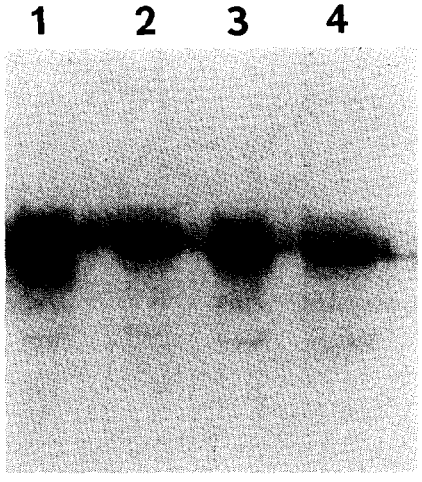

Fig. 2. Increased synthesis of dehydratase protein by gyrase inhibitors. A Western blot of lysates prepared from cells incubated in TYE with 5 μg/ml of CM (lane 1), 0.05% dimethyl sulfoxide used as solvent for CM (lane 2), 10 μg/ml NA (lane 3) and no inhibitor (lane 4).

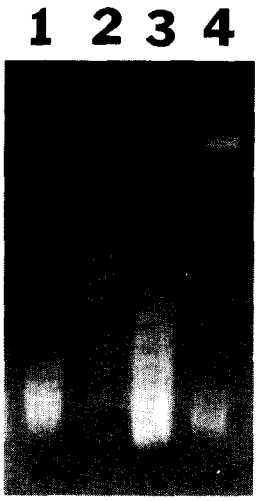

Fig. 3. Effect of CM on the extent of DNA supercoiling. Plasmid DNAs isolated from cells incubated anaerobically for 5 h with 0.05% dimethyl sulfoxide control (lane 1), and 5 μg/ml of CM (lane 2). Lanes 3 and 4 contained pBR322 DNA isolated from aerobically and anaerobically grown cells respectively in the absence of gyrase inhibitors.

cells were incubated with novobiocin the extent of DNA supercoiling was reduced. The data presented in Fig. 3 show that a reporter plasmid pBR322 DNA isolated from K37 incubated anaerobically with CM showed less DNA supercoiling (lane 2) as compared to plasmid DNA isolated from a control culture without CM (lane 1). Lanes 3 and 4 depict the extent of supercoiling of pBR322 DNA isolated from aerobically and anaerobically grown cells respectively. In these same cultures CM stimulated dehydratase synthesis by about 3-fold.

5. DISCUSSION

The experiments summarized here indicate that the specific activity of threonine dehydratase is stimulated several-fold by gyrase inhibitors. Immunoblot with antidehydratase antibodies shows increased synthesis of the dehydratase protein and the effect of rifampicin suggests that continued synthesis of *tdc* mRNA is needed for de novo synthesis of threonine dehydratase. Because *tdcB* is the second gene of the *tdc* operon which is

transcribed from a single functional promoter located upstream of *tdcA* [6,7], it is likely that gyrase inhibitors influence the expression of all three genes belonging to the *tdc* operon.

The biochemical mechanism of increased transcription of the *tdc* genes by gyrase inhibitors remains to be established. Dorman et al. [5] found a direct correlation between relaxation of supercoiled DNA and, the anaerobic and growth phase-dependent, *tonB* expression. The results displayed in Fig. 3 clearly show that the coumermycin-mediated stimulation of threonine dehydratase synthesis was also accompanied by relaxation of DNA supercoiling. These data are interpreted to mean that a decreased level of DNA gyrase activity in vivo, which results in less supercoiled DNA, facilitates transcription of the *tdc* genes. Continued investigation of the regulation of the *tdc* operon would provide further insight into the global control of anaerobic gene expression influenced by subtle changes in topology of chromosomal DNA.

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