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Effect of DNA Gyrase Inhibitors on Gene Expression of the Cysteine Regulon

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Summary. Nalidixic acid inhibits the expression of those cysteine genes which are regulated by the *cysB* product, it has no affect, however, on the constitutively expressed *cysE* gene. The expression of cysteine genes in a strain carrying a mutation in the *nalA* locus is resistant to this drug. Novobiocin affects the expression of cysteine genes similarly to nalidixic acid. The effect of nalidixic acid on the expression of genes in a cysteine constitutive mutant was studied.

Introduction

Nalidixic acid has long been known as an inhibitor of DNA synthesis (Goss et al. 1965). The mechanism of the inhibitory action of this drug on nucleic acid synthesis was clarified by the discovery and characterization of DNA gyrase (Gellert et al. 1976a). DNA gyrase catalyses the ATP-dependent introduction of negative superhelical twists into double-stranded closed circular DNA and the ATP-independent relaxation of superhelical DNA. The activity of DNA gyrase is inhibited by two families of antibiotics, one represented by novobiocin and coumermycin A, the other by nalidixic acid and oxolinic acid (Gellert 1976b). Mutants resitant to each of these types of drug are known. The nicking-closing activity required in the supercoiling reaction depends on *nalA* gene product (Gellert et al. 1979). Several workers have also observed that, at higher doses, these antibiotics affect transcription (Puga and Tessman 1973; Falco et al. 1978). Recently it has been found that read-through transcription of the *trp* operon from the leftward φ80p*trp* promoter is sensitive to gyrase inhibitors, whereas transcription from the *trp* promoter is gyrase-independent (Smith et al. 1978). These results, as others, (Flashner et al. 1977; Mandel and Chambon 1974) suggest the dependence of promoter activity on supertwist density. The facilitation of RNA polymerase binding by negative superhelical turns may be of varying importance for different promoters. Shumman and Schwartz (1975) reported that nalidixic acid inhibits the expression of catabolic sensitive operons. This observation was confirmed in the work of Sanzey (1979). This author observed a correlation between the inhibitory effect of this antibiotic on the operon expression and the type of operon control: transcription of operons that are at least partly positively regulated and that require a functional cAMP receptor protein was inhibited by nalidixic acid.

It was of interest to check whether nalidixic acid and novobiocin affect the expression of the cysteine regulon, a positively regulated anabolic pathway, in which a functional product of the *cysB* gene is necessary for the expression of cysteine biosynthetic genes with the exception of *cysE* and *cysG* (Kredich 1971; Ostrowski and Hulanicka 1979).

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Materials and Methods

Media and Culture Conditions. Media and cultures have been described elsewhere (Hulanicka et al. 1979).

Strains. All strains were nonlysogenic derivatives of *S. typhimurium* LT-2 and are listed in Table 1.

Isolation of nalA Mutants. Spontaneous Nal-resistant mutants *nal* of strain TK1000 were isolated by plating at 37° C about 10^8 cells on complete medium supplemented with 20 μg of Nal per ml. The corresponding *nalA* mutations were identified by cotransduction with the *glpT* locus by phage P22 transduction.

Enzyme Assays. O-acetylserine sulfhydrylase, and serine transacetylase were determined as described previously (Kredich 1971), sulfite reductase was assayed by the method of Vito and Dreyfuss (1964). Protein was determined by the biuret method using bovine serum albumin as standard (Gornal et al. 1949).

Chemicals. O-Acetyl-L-serine was prepared by the method of Sakami and Toennies (1942). Nalidixic acid and novobiocin were purchased from Sigma. All other chemicals were commercial products of chemical reagent grade.

Table 1. Designation and derivation of *Salmonella typhimurium* strains

Strain	Genotype	Source or method of construction
TK1000	Wild-type	N.D. Zinder
TK2153	*nalA*	mutation in strain TK1000
TK2154	*glpT*	mutation in strain TK1000
TK2120	*ara9 hisC340 thy cysB^{c}2346*	transductant from TK2119 lysate × DW221
TK2167	*leu500 (supX cysB1763) pyrF146 cys2332*	mutation in strain DW363
DW363	*leu500 (supX cysB1763) pyrF146*	N.M. Kredich

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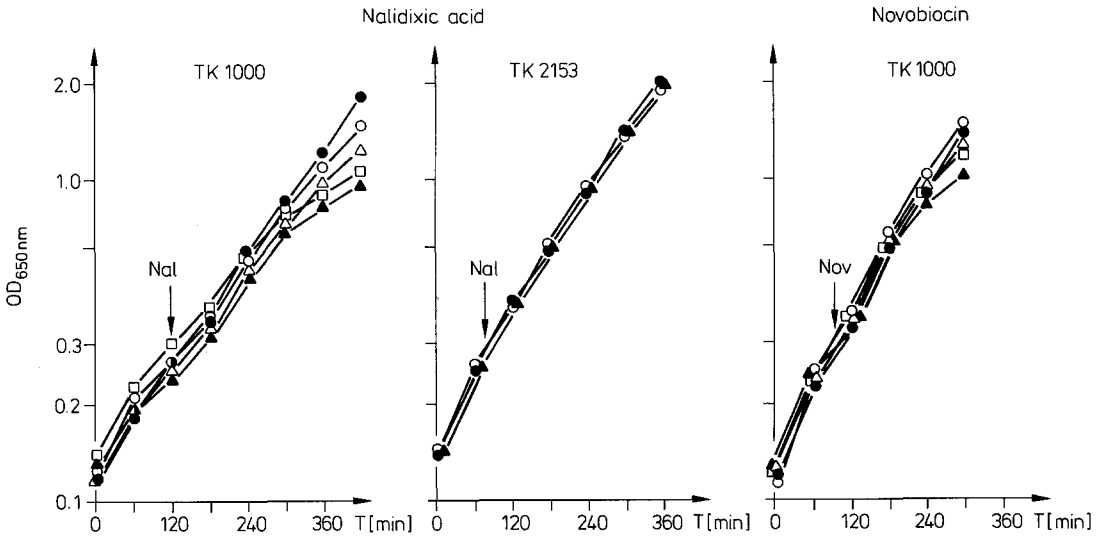

Fig. 1. Effect of the different inhibitors of DNA gyrase on bacterial growth. Minimal medium supplemented with: nalidixic acid (Nal). ●—● 0 µg/ml; ○—○ 10 µg/ml; △—△ 20 µg/ml; □—□ 40 µg/ml; ▲—▲ 60 µg/ml, or novobiocin (Nov) ●—● 0 µg/ml; ○—○ 50 µg/ml; △—△ 100 µg/ml; □—□ 200 µg/ml; ▲—▲ 350 µg/ml

Table 2. Effect of nalidixic acid on the induction of sulfite reductase, O-acetylserine sulfhydrylase and serine transacetylase

Strain	Relevant genotype	Conc. of Nal µg/ml	SR µg/ml prot.	% of control	OASS µ/mg prot.	% of control	STA mµ/mg/prot.	% of control
TK1000	*nalA*$^+$	0	185	100	10	100	25	100
		10	56	30	5	50	23	92
		20	44	24	4	40	24	96
		40	28	15	3	30	28	112
		60	26	14	2	20	25	100
TK2153	*nalA*	0	189	100	11	100	28	100
		10	194	103	11	100	30	107
		20	191	101	10	91	27	96
		40	200	106	14	127	29	104
		60	219	115	10	91	28	100

SR – sulfite reductase; OASS – O-acetylserine sulfhydrylase; STA – serine transacetylase

Results

Effect of Nalidixic Acid on Expression of Cysteine Genes

Strain TK1000 of *Salmonella typhimurium* which is sensitive to nalidixic acid was used in the experiments. Overnight inocula grown on cysteine were resuspended in BS medium containing L-djenkolic acid. Different concentrations of nalidixic acid were added when the absorbance at 650 nm reached 0.3 (Fig. 1). The concentration of nalidixic acid used (up to 60 µg/ml) hardly affects the further increase of cell mass during the experiments. DNA replication measured by the incorporation of radioactive thymidine in the presence of deoxyadenosine, was immediately inhibited, however (data not shown). Bacteria were harvested at an absorbance of 0.5 and cell-free extracts were assayed for cysteine biosynthetic enzymes: sulfite reductase, O-acetylserine sulfhydrylase and serine transacetylase. Two branches of the reaction pathway lead to biosynthesis of cysteine. One arm consists of the assimilation reaction and reduction of sulfate, the second provides the carbon skeleton of cysteine. The final step of L-cysteine biosynthesis in *Salmonella typhimurium* and *Escherichia coli* consists of the sulfhydrylation of O-acetyl-L-serine by sulfide. All the enzyme of the reductive branch of the cysteine biosynthetic pathway are coordinately regulated (Spencer et al. 1967). Sulfite reductase can be considered as a representative of the sulfate assimilation branch. The results presented in Table 2 indicate that nalidixic acid inhibits the expression of sulfite reductase and O-acetylserine sulfhydrylase, whereas it has no effect on the level of serine tansacetylase, which is the product of the *cysE* gene. Even at a concentration as low as 10 µg/ml the drug caused significant inhibition, the levels of sulfite reductase and O-acetylserine sulfhydrylase being 30% and 50% respectively of the control specific activity. The activity of serine transacetylase was not affected by the presence of nalidixic acid. Higher concentrations of nalidixic acid resulted in a slightly higher inhibition of the expression of sensitive genes. The effect of concentrations of nalidixic acid higher than 60 µg/ml could not be examined since bacterial growth was severely affected.

In further experiments the effect of nalidixic acid on resistant strains was studied. The same concentration of the drug has no effect on the level of the enzymes studied in the *nalA* strain (TK2153) (Table 2). The above results indicate that *nalA* gene product is involved in the transcription of these genes which are under positive control of the *cysB* product, whereas the expression of the *cysE* gene, which is independent of the *cysB* product, is insensitive to this drug.

Table 3. Effect of novobiocin on induction of sulfite reductase and O-acetylserine sulfhydrylase in strain TK1000

Concentration of novobiocin μg/ml	SR μg/mg prot.	% of control	OASS μ/mg prot.	% of control
0	194	100	10	100
50	157	81	9	90
100	139	72	5	50
200	110	57	4	40
350	67	34	3	30

SR – sulfite reductase; OASS – O-acetylserine sulfhydrylase

Table 4. Effect of nalidixic acid on the induction of sulfite reductase and O-acetylserine sulfhydrylase in cysteine constitutive mutants

Strain	Relevant genotype	Conc. of Nal μg/ml	SR μg/mg prot.	% of control	OASS μ/mg prot.	% of control
TK2120	*cysB*^{c}*2346*	0	120	100	14	100
		10	56	47	9	64
		20	31	26	4	29
		40	28	23	3	21
		60	25	21	2	14
TK2167	*cys2332*	0	25	100	–	–
		10	27	108	–	–
		40	27	108	–	–

SR – sulfite reductase; OASS – O-acetylserine sulfhydrylase

Effect of Novobiocin on the Expression of Sulfite Reductase and O-Acetylserine Sulfhydrylase

It was important to check whether the second subunit of the gyrase is also involved in regulation of gene expression. We tested whether the antibiotic novobiocin, a specific inhibitor of this subunit, affects the expression of *cys* genes in the same way as nalidixic acid. The addition of novobiocin to the culture showed the same effect as nalidixic acid. The levels of two enzymes, sulfite reductase and O-acetylserine sulfhydrylase, were decreased (Table 3) whereas the specific activity of serine transacetylase was the same as in the control culture (data not shown). Therefore, the whole DNA gyrase complex seems to be involved in the expression of positively regulated cysteine genes.

Sensitivity of Cysteine Constitutive Mutants to Nalidixic Acid

Mutation in a promoter might alter the sensitivity of a given operon to nalidixic acid. Two types of cysteine constitutive mutants have been described, *cysB*^{c} strains and *cys2332* the constitutive mutant of the *cysJIH* operon. (Kredich 1971; Sledziewska and Hulanicka 1978; Ostrowski and Hulanicka 1979). The *cysB*^{c} constitutive mutants are characterised by a depressed unregulated level of cysteine enzymes. In these mutants, the expression of the cysteine genes is independent of the presence of O-acetyl-L-serine, the internal inducer of the cysteine pathway. The expression of the *cysJIH* operon, in the *cys2332* strain is independent of the presence of the regulatory protein and O-acetyl-L-serine.

The effect of nalidixic acid on these two types of constitutive strains was studied. The results presented in Table 4 show that nalidixic acid inhibits only the expression of the cysteine genes in *cysB*^{c} strains, whereas it has no effect on the expression of the *cys2332* mutant. In the case of the *cys2332* strain the activity of O-acetyl-L-serine sulfhydrylase could not be checked, since this strain carries a *cysB* deletion. Table 4 presents the results of experiments with TK2120 (*cysB*^{c}*2346*) as a representative *cysB*^{c} constitutive mutant. Similar results were obtained with other constitutive strains.

Discussion

Recently, a number of observations have been made indicating inhibition of transcription by inhibitors of DNA gyrase such as nalidixic acid and novobiocin. The degree of supercoiling of DNA seems to play a role in transcription regulation (Peebles et al. 1979). They suggest that negative supercoiling facilitates RNA polymerase binding and this may be of varying importance for different promoters. Huey-Lang Yang et al. (1979) investigated the effect of DNA gyrase inhibitors on gene expression and they found that it depends on the structure of the DNA template. Sanzey (1979) published a paper in which she demonstrated that DNA gyrase is involved in the recognition of catabolite sensitive promoters by RNA polymerase. The results presented in this paper show that nalidixic acid and novobiocin inhibit the expression of those cysteine genes which are under the control of the *cysB* locus. The expression of the *cysE* gene, which belongs to the cysteine regulon but is constitutively expressed, is not affected by the inhibitors (Table 2). Similar results were obtained with *E. coli* strains (data not shown). The lack of effect of nalidixic acid on the expression of the studied genes in the *nalA* mutant (Table 3) and its inhibition of gene expression in the wild-type *nalA*$^+$ strain indicate that the observed effects were caused by inhibition of gyrase activity. The results of the experiments with cysteine constitutive mutants showed that the *cys2332* mutation renders the expression of sulfite reductase genes insensitive to nalidixic acid. The *cys2332* mutation probably affects initiation of transcription and may modify the structure of the promoter region of the *cysJIH* operon so that gyrase supercoiling activity, is not necessary for facilitation of RNA polymerase binding. The constitutivity of the *cysB*^{c} mutant results from the altered *cysB* product. In this mutant the expression of cysteine genes is independent of the presence of O-acetyl-L-serine and the sulfur sources. The regulatory regions of the cysteine structural genes are not changed and the expression of genes in these mutants is still sensitive to the inhibitors of DNA gyrase. The requirement for gyrase activity for expression of some positively controlled operons suggests that negative superhelicity facilitates the binding of activator protein.

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