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Indolmycin-Mediated Inhibition and Stimulation of Transcription at the *trp* Promoter of *Escherichia coli*†

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Escherichia coli cells harboring a non-attenuated *trp-lac* operon fusion were used to evaluate the effects of indolmycin on the initiation of transcription at the *trp* promoter. Indolmycin caused repression in *trpR*$^+$ strains and in *trpR* deletion mutants, although higher effector concentrations were required in the latter situation. Plasmid-mediated elevation in tryptophanyl-tRNA synthetase reversed the inhibitory effect of indolmycin. Indolmycin did not facilitate the binding of purified Trp repressor protein to *trp* operator DNA.

Indolmycin, originally designated antibiotic PA155A (8, 12), was isolated from a streptomycete subsequently identified as *Streptomyces griseus* (Krainsky) Waksman and Henrici sensu Pridham (13). Elucidation of the structure of indolmycin (14), supported by its complete chemical synthesis (10), revealed the presence of an indole nucleus (Fig. 1). Indeed, studies on the biosynthesis of indolmycin indicate that it is derived from tryptophan (6, 7). Detailed chemical studies have been performed on indolmycin, and its absolute configuration is known (2, 15).

The first study of the mechanism of action of indolmycin revealed that it is a competitive inhibitor of tryptophanyl-tRNA synthetase of *Escherichia coli* (17). Recent experiments have shown that at least one class of *E. coli* mutant resistant to indolmycin has a tandem duplication of *trpS*, the structural gene for tryptophanyl-tRNA synthetase (R. Bryant, K. B. Low, and D. Soll, personal communication). Presumably, the increased *trpS* gene dosage leads to elevated intracellular levels of tryptophanyl-tRNA synthetase. Resistance to indolmycin in this case would be attributable to a specific increase in cellular capacity to aminoacylate $tRNA^{Trp}$. The in vivo studies presented here with specially constructed *E. coli* strains largely confirm these findings. In addition, we have found that indolmycin can lead to a reduction in the expression of the *trp* operon by acting as a false corepressor.

A pair of essentially isogenic strains was utilized. NK5031 (Table 1) carries an intact *trpR* gene (the structural gene for Trp repressor), whereas SP361 has the entire *trpR* gene deleted. These strains were lysogenized with λGB2 (Table 1), a phage wherein the primary *trp* promoter-operator (but no attenuator) is fused to *lacZ*, the structural gene for β-galactosidase. Both NK5031 and SP361 are deleted for *lacZ*; thus, in λGB2 lysogens, the levels of β-galactosidase reflect transcription initiated at the *trp* promoter. Repression by exogenous L-tryptophan in such strains conformed to the predicted pattern. The levels of β-galactosidase in the strain capable of producing functional Trp repressor were reduced by a factor of 25 (from 3,980 to 160 units) when tryptophan was present (see the legend to Fig. 2 for the definition of units of β-galactosidase). The strain deleted for *trpR* had β-galactosidase levels after growth in media containing tryptophan (6,520 U) that were essentially indistinguishable from the values (6,470 U) obtained after cultivation in tryptophan-free media. Repression by exogenous tryptophan was not altered by the presence of a multicopy *trpS*$^+$ plasmid.

A high-copy number plasmid, designated ptrpS13 (Table 1), was constructed and introduced into each λGB2 lysogen. ptrpS13 is identical in all respects to pCH17 (5), a plasmid for which nucleotide sequencing studies have confirmed the presence of the entire *trpS* gene (4). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of extracts of cells harboring ptrpS13 confirm that tryptophanyl-tRNA synthetase (monomer molecular weight, 37,000) is overproduced in these strains (C. Makaroff and R. Somerville, unpublished data). Strains harboring other plasmids similar to ptrpS13 exhibit levels of tryptophanyl-tRNA synthetase 55-fold higher than those in wild-type strains (J. Omnaas, M. Bonura, P. Chang, P. A. Safille, and K. H. Muench, Fed. Proc. 38:821, 1979).

The effect of indolmycin on transcription from the *trp* promoter is shown in Fig. 2. With cells capable of producing Trp repressor, low concen-

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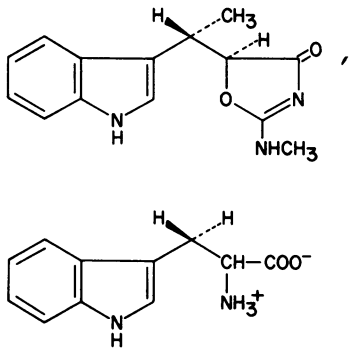

FIG. 1. Structures of indolmycin (top) and tryptophan (bottom).

trations of indolmycin (0.01 μg/ml) markedly decreased transcription from the *trp* promoter. When tryptophanyl-tRNA synthetase levels were elevated or when Trp repressor was absent, reductions in *trp* promoter utilization were observed only at high concentrations (>0.5 μg/ml) of indolmycin. Finally, when both tryptophanyl-tRNA synthetase levels were elevated and Trp repressor was absent, the *trp* promoter was completely unresponsive to very high levels of indolmycin. This was true even at 200 μg of indolmycin per ml (data not shown).

All of the strains studied were totally unaffected with respect to growth rate over a range of indolmycin concentrations from 0 to 1.0 μg/ml. From 1.0 to 5.0 μg of indolmycin per ml, the strains harboring ptrpS13 were unaffected, but the non-plasmid-bearing strains exhibited a slight growth inhibition. Except for SP361(λGB2)(ptrpS13), the strains displayed marked sensitivity to levels of indolmycin above 5 μg/ml.

The levels of β-galactosidase in the four strains studied were essentially identical (plus or minus 7%) regardless of whether the assays were carried out on cultures entering stationary phase or involved differential rate determinations from cultures sampled during mid-log phase. For example, the relative activity of β-galactosidase from a near-stationary phase culture of NK5031 (λGB2) in 0.01 μg of indolmycin per ml was 62% of the control value, whereas a differential rate analysis of mid-log phase cells gave a value of 65% of the control value (Fig. 2).

In the presence of Trp repressor protein, indolmycin mediated repression of the *trp* promoter most effectively at a concentration of 1 μg/ml (Fig. 2). At this level of indolmycin, β-galactosidase production in a *trpR* deletion was totally unaffected, whereas the activity in the *trpR*$^+$ strain was nearly undetectable.

Tryptophan readily mediated the in vitro binding of Trp repressor protein to the *trp* operator, but indolmycin did not (Fig. 3). Thus, a paradox seems to exist. In whole cells capable of making Trp repressor, indolmycin reduced transcription from the *trp* promoter, yet this compound failed to mediate in vitro the binding of Trp repressor to its primary DNA target.

The data in Fig. 2 reveal two further complications. First, at concentrations of indolmycin above 1 μg/ml, reduction of transcription initiation at the *trp* promoter occurred even in the absence of Trp repressor (though not when tryptophanyl-tRNA synthetase levels were elevated). Second, in *trpR*$^+$ cells with elevated levels of tryptophanyl-tRNA synthetase, low concentrations of indolmycin (up to 0.2 μg/ml) actually increased β-galactosidase activity. The basis for these observations is unclear, although it may be concluded that the *trp* promoter is subject to stimulation and inhibition at certain concentrations of indolmycin via mechanisms that need not involve the participation of Trp repressor.

One explanation of these results might be that indolmycin acts indirectly to inhibit the transla-

TABLE 1. Bacterial strains, phage, and plasmids

Group	Genotype	Relevant property	Source or reference
Strains			
NK5031	Δ(*lacZ*)*MM5265 gyrA supF*	*trpR*$^+$ present	N. Kleckner via K. Bertrand
SP361	Δ(*serB-trpR*)*37-1* Δ(*lacZ*)*MM5265 gyrA supF*	*trpR* deleted	1
Phage			
λGB2	*lacZ*$^+$ *bet gam* cI857 *nin5 S*$^+$	*trp* operon promoter-operator (but no attenuator) fused to *lacZ*	R. Somerville and G. Bogosian, manuscript in preparation
Plasmids			
ptrpS13	*trpS*$^+$ *bla*$^+$	Tryptophanyl-tRNA synthetase overproducer	This work
pPS21		Source of 570-base-pair *Hpa*II fragment for operator protection studies (Fig. 3)	9

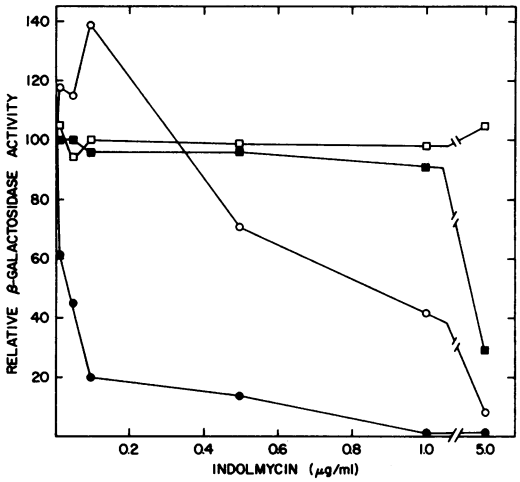

FIG. 2. Effect of indolmycin on expression of β-galactosidase from a *trp-lacZ* fusion. β-Galactosidase assays were performed as described previously (3). For the assays, cultures were grown with shaking to mid-log phase at 30°C in 10 ml of Vogel and Bonner minimal media (16) supplemented with (per ml) 1 μg of thiamine hydrochloride, 0.1 μg of biotin, 2 mg of acid casein hydrolysate, and 2 mg of glycerol, plus indolmycin as indicated. Crystalline indolmycin (lot no. 10428-158-1) was a gift from R. C. Koch, Pfizer Inc., Groton, Conn. This material was shown by bioassay to be tryptophan-free. Symbols: ■, SP361(λGB2); □, SP361(λGB2)(ptrpS13); ●, NK5031(λGB2); ○, NK5031(λGB2)(ptrpS13). Initial (no indolmycin) values were 6,520 U [SP361(λGB2)], 7,290 U [SP361(λGB2)(ptrpS13)], 3,980 U [NK5031(λGB2)], and 2,980 U [NK5031(λGB2)(ptrpS13)]. The specific activity units are 200 nmol of *o*-nitrophenol liberated per ml of culture per absorbance unit at 660 nm per min.

tion of β-galactosidase mRNA. Under conditions of limited tryptophan biosynthetic capability, competitive inhibition of tryptophanyl-tRNA synthetase by indolmycin could curtail translation. Such complications would presumably not apply in the absence of Trp repressor or when tryptophanyl-tRNA synthetase levels were elevated. This hypothesis was ruled out, however, by the fact that a *pheA-lacZ* fusion was completely unresponsive to indolmycin (in vivo data not shown). Nor was the *trpR* promoter, a known target of Trp repressor (1), affected by indolmycin (in vivo data not shown), implying that indolmycin represses the *trp* operon promoter in a specific fashion.

Another possibility is that indolmycin-mediated inhibition of tryptophanyl-tRNA synthetase leads to increased intracellular levels of free tryptophan, thereby potentiating the activity of Trp repressor. Our finding that indolmycin-directed repression is specific for the *trp* operon and does not affect other known targets of Trp repressor, such as the *trpR* promoter, may not necessarily contradict this hypothesis. Trp repressor is known to have a higher affinity for the operator preceding the *trp* operon than for the operator preceding *trpR* (1). This hypothesis fails to explain both the indolmycin-mediated increase in *trp* operon expression noted in *trpR*$^+$ cells with elevated levels of tryptophanyl-tRNA synthetase and the decrease in *trp* operon expression that indolmycin can bring about in the absence of Trp repressor (Fig. 2).

Finally, it is also conceivable that an indolmycin-tryptophanyl tRNA synthetase complex influences the initiation of transcription at the *trp* promoter (11).

Clearly, the results presented here are not readily explained by current ideas about the control of transcription initiation at the *trp* promoter (18). Indolmycin appears to specifically inhibit transcription from the *trp* promoter by mechanisms that are enhanced in *trpR*$^+$ cells and countered by plasmid-mediated increases in intracellular tryptophanyl-tRNA synthetase. In addition, under certain conditions (Fig. 2), transcription at the *trp* promoter can be enhanced as much as 40% by indolmycin.

It should be stressed that attenuation does not have a role in indolmycin-mediated repression

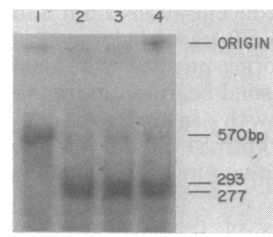

FIG. 3. Indolmycin does not facilitate the binding of Trp repressor protein to *trp* operator DNA. A 570-base-pair *Hpa*II fragment carrying the primary *trp* promoter operator region was isolated from plasmid pPS21. The fragment was dephosphorylated with calf intestinal alkaline phosphatase, and the *Hpa*II ends were labeled with ^{32}P, using T4 polynucleotide kinase (Bethesda Research Laboratories [BRL]). Approximately 0.1 μg of end-labeled fragment was mixed with enough purified Trp repressor protein to completely protect the *Hpa*I site within the *trp* operator from digestion in the presence of L-tryptophan. Reactions were in a total volume of 20 μl containing 20 mM Tris-hydrochloride (pH 7.5), 10 mM $MgCl_2$, 1 mM dithiothreitol, and 50 mM KCl. Indolmycin was added from a 4-mg/ml stock solution in dimethyl sulfoxide. After 30 min at 37°C, 1 U (1 μl) of *Hpa*I (BRL) was added, and incubation continued for an additional 30 min. Products were separated on a 5% acrylamide gel. Lanes: (1) 100 μg of L-tryptophan per ml present; (2) no added tryptophan; (3) +200 μg of indolmycin per ml; (4) +400 μg of indolmycin per ml.

or stimulation. The *trp-lacZ* fusion employed in these studies was so constructed that the entire *trp* attenuator was absent. These results suggest a direct role for tryptophanyl-tRNA synthetase in the regulation of the *trp* operon in addition to its well-established indirect role in attenuation (18). Elucidation of the mechanism of indolmycin-mediated alteration of transcription from the *trp* promoter may reveal unanticipated features of control in the *trp* system.

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