Transcription Termination Factor rho: The Site of Bicyclomycin Inhibition in *Escherichia coli*†

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ABSTRACT: Bicyclomycin is a novel, commercially important antibiotic. Information concerning the site of bicyclomycin inhibition in *Escherichia coli* has been obtained by the production of bicyclomycin resistant mutants by UV irradiation. Selection by growth in the presence of bicyclomycin of a plasmid clone library generated from a highly resistant mutant in recipient antibiotic-sensitive host cells (*E. coli* strain W3350) has led to the characterization of three different plasmids that confer drug resistance, which contained the gene encoding the transcription termination factor, rho. These mutant rho genes contained single base changes at nucleotide positions 656, 796, and 1009. Preliminary mechanistic information has been obtained by monitoring the polyC-dependent ATPase activity of rho in the absence and presence of bicyclomycin and dihydrobicyclomycin. Addition of bicyclomycin to aqueous solutions containing rho and ATP led to a decrease in the release of inorganic phosphate with an I_{50} value of 60–70 μM bicyclomycin. This inhibition is comparable to the drug concentration needed to inhibit bacterial growth on plates. No loss of activity was observed when a similar concentration of dihydrobicyclomycin was used in place of bicyclomycin, while use of 10-fold higher concentrations of this derivative led to partial rho inhibition. PolyC-dependent ATPase activity from partially purified rho isolated from the mutant BCMr108 was not inhibited by bicyclomycin at concentrations (200 μM) found to completely inhibit wild-type rho. These cumulative findings are consistent with the notion that bicyclomycin expresses its activity by interfering with the polyC-dependent ATPase activity of rho.

The increasing occurrence of organisms resistant to conventional antibiotic therapies has prompted the search for new antibacterial agents having novel modes of action. Bicyclomycin (Figure 1) is a structurally unique, commercial antibiotic (Miyoshi et al., 1972; Myamura et al., 1972) possessing a broad spectrum of antimicrobial activity against Gram-negative bacteria (Miyoshi et al., 1972). Considerable controversy exists concerning the mode of action of bicyclomycin (Abuzar & Kohn, 1990). Previous studies have suggested that the antibiotic affects bacterial outer-membrane synthesis. Several findings have contributed to this notion. First, administration of bicyclomycin to *Escherichia coli* led to modified cell wall synthesis (Someya et al., 1978; Pisabarro et al., 1986). Second, decreases in lipoprotein concentrations were observed in the presence of bicyclomycin (Martin et al., 1974). Third, administration of the antibiotic led to changes in the proportion of peptidoglycan linkages (Pisabarro et al., 1986). Fourth, several outer-membrane proteins differing from those interacting with penicillin were found to bind [^{14}C]-bicyclomycin (Someya et al., 1978). Additional advances, however, have been stymied by the inability to conclusively identify the site(s) of bicyclomycin action.

Here we report that bicyclomycin directly affects the polyC-dependent ATPase activity of the transcription termination factor rho in *E. coli* (Das et al., 1978; Platt, 1986; Richardson, 1990; Yager & von Hippel, 1987). This finding places bicyclomycin in a category with rifamycin B and actinomycin D, two other antibiotics known to be involved with RNA transcriptional processes (Stryer, 1988). Significantly, the mechanisms of action for these three agents are distinctly different.

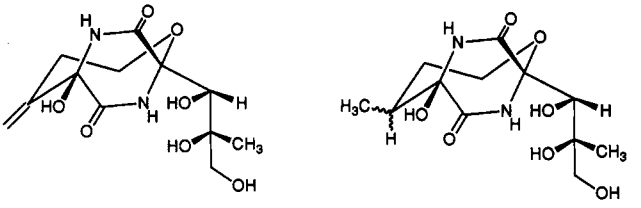

FIGURE 1: Structures of bicyclomycin and dihydrobicyclomycin.

METHODS AND MATERIALS

Bacterial Strains and Plasmids. The *E. coli* W3350 and host ER1451 were grown on LB[1] media, and bicyclomycin-resistant mutants were grown on LB media supplemented with 200 μg/mL bicyclomycin. Plasmid pBGS18, conferring kanamycin resistance selection, was used as the subcloning vector. Bicyclomycin was provided as a gift from Fujisawa Pharmaceutical Co., Ltd., and all other chemicals and antibiotics were of the highest quality obtainable.

Bicyclomycin Resistance Assays. Select strains or host cells carrying recombinant plasmids were grown in LB broth, and serial dilutions were performed. Aliquots of the serial dilutions of cells were plated on LB plates containing concentrations of bicyclomycin varying from 5 to 1000 μg/mL; the samples were plated in triplicate. Colonies were counted after overnight growth at 37 °C.

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[1] Abbreviations: ATP, adenosine triphosphate; I_{50}, 50% inhibition; UV, ultraviolet; LB, Luria broth; BCMr, bicyclomycin resistance; CTAB, cetyltrimethylammonium bomide; SDS, sodium dodecyl sulfate.

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UV Mutagenesis. Wild-type *E. coli* W3350 (genotype) cultures were grown on LB broth to mid-to-late log stage growth. From a 50-mL culture containing 3×10^9 cells/mL, 10 mL was transferred to a sterile Petri dish and exposed to UV radiation from a 30-W Sylvania Germicidal lamp 30T8 from 15 cm for 50 s, at which time about 5–10% survival was seen. Cells were concentrated by centrifugation and resuspended in 1 mL, from which 200-μL aliquots were spread on LB plates containing 200 μg/mL bicyclomycin. The plates were incubated overnight at 37 °C, and bicyclomycin-resistant colonies were replated on LB plates supplemented with 200 μg/mL bicyclomycin.

Isolation of Genomic DNA. The bicyclomycin-resistant mutant, BCMr108, was grown overnight in 500 mL of liquid LB broth containing 200 μg/mL bicyclomycin. Cells were washed and resuspended in STE buffer (10 mM NaCl, 20 mM Tris-HCl, pH 8.0, 1 mM EDTA). DNA was isolated by published methods (Dillela & Woo, 1985) with modifications. After a second proteinase K–SDS digestion, the DNA was subjected to CTAB extraction (Ausubel et al., 1989). The resultant DNA was suspended in 4 mL of 1× TE buffer.

Partial Sau3AI DNA Digestion. Four aliquots of DNA containing 400 μg in 4 mL (i.e., 0.1 mg/mL) were digested with 20 units of *Sau*3AI at 37 °C for 5, 10, 15, and 20 min. The digestions were stopped by the addition of EDTA to 50 mM, and the samples were placed on ice. The aliquots were pooled and precipitated by the addition of 0.3 M sodium acetate and 16 mL of isopropyl alcohol. The DNA was placed on 10–40% sucrose gradients and centrifuged using a Beckman SW-41 rotor at 35 000 rpm for 20 h (Ausubel et al., 1989). Fractions (0.5 mL) were collected, and the fragment sizes were determined by agarose gel electrophoresis. Fractions containing sized DNA in the range of 2–6 kbp were pooled, precipitated, and redissolved in 1× TE buffer.

Screening for Bicyclomycin Resistance. The DNA was ligated into *Bam*HI-linearized pBGS18 plasmid, which codes for kanamycin resistance. Transformed cells were plated out on LB plates containing 50 μg/mL kanamycin. After overnight growth, colonies were transferred by filter colony lifts onto plates containing 200 μg/mL bicyclomycin and allowed to grow for 2 days. The colonies showing bicyclomycin resistance were picked and restreaked on fresh bicyclomycin plates. Single colonies were chosen for growth in liquid cultures for further characterization.

DNA Sequencing. The plasmid DNA was subcloned into M13mp18/19, and DNA sequencing was performed using standard dideoxy chain termination techniques (Sanger et al., 1977) as described in the Sequenase kit (U.S. Biochemical, Cleveland, OH). Genbank was searched using the UWGCG (University of Wisconsin genetics computer group) program FASTA (Devereux et al., 1984) on a 3100 VAX workstation connected to a 3300 file server.

Growth Curves. Rates of growth of mutants and plasmid-bearing strains containing the bicyclomycin-resistant phenotype were measured as the increase in optical density at 550 nm; they were grown at 37 °C in a shaking water bath. Plasmid strains grown first in liquid cultures containing kanamycin were transferred to liquid cultures containing 200 μg/mL bicyclomycin.

PCR Amplification of Wild-Type and Bicyclomycin-Resistant rho Transcription Termination Gene. Genomic DNA isolated from the other bicyclomycin-resistant mutants and W3350 were amplified using primers designed at 29 bp upstream of the rho coding frame and 742 bp downstream from the termination codon after the *Hind*III site. The product, amplified using standard protocols (Saiki et al., 1985) as supplied by the manufacturer (Cetus, Perkin-Elmer), was digested with *Bcl*I and *Hind*III and ligated into pBGS18. Transformed cells were first selected on kanamycin and picked onto bicyclomycin-containing plates. Restriction digests using either *Eco*RI–*Hind*III or *Eco*RI were employed to confirm the presence of the correct plasmid containing the rho gene.

Isolation of rho Protein. Strain AR120 containing the overproducing plasmid p39-AS was grown in LB to 1.0 OD, and nalidixic acid was added to a concentration of 40 μg/mL. The culture was allowed to incubate at 37 °C for 4 h, and rho was isolated following published methods (Finger & Richardson, 1981) modified according to Sharp et al. (1983). Briefly, frozen cell pellets were thawed and disrupted in 0.05% sodium deoxycholate and then treated with polymin P. The protein was precipitated with ammonium sulfate, dialyzed, and chromatographed on a Bio-Rex 70 column. rho was eluted from the column with a linear salt gradient. Fractions showing rho activity were pooled and concentrated. rho protein purity was determined by SDS–PAGE electrophoresis, and protein concentrations were determined by published methods (Lowry et al., 1951). rho protein was dialyzed against storage buffer (10 mM Tris-HCl, pH 8.0, 50% glycerol, 100 mM NaCl, 0.1 mM EDTA, 0.1 mM DTT) and frozen in aliquots. A comparable protocol was employed for the isolation of mutant rho from the strain BCMr108, with the exception that larger starting volumes of cells were used and the nalidixic acid induction step was omitted.

PolyC-Stimulated ATPase Activity. The polyC-dependent ATPase activity of rho at 32 °C was assayed by two independent methods. The first method (Das et al., 1978) measured the release of [^{32}P]phosphate from [γ-^{32}P]ATP in the presence and absence of added bicyclomycin or dihydrobicyclomycin (Figure 1; Kamiya et al., 1972) by silicotungstate–molybdate complexation and extraction in benzene/isobutyl alcohol followed by liquid scintillation counting. The second method (Sharp et al., 1983) measured the amount of hydrolyzed [^{32}P]phosphate after separation on Baker-Flex cellulose PEI TLC plates (J. T. Baker, Inc., Phillipsburg, NJ), using 0.75 M potassium phosphate, pH 3.5, as the mobile phase. The TLC plates were exposed to XAR5 X-ray film, and the bands visualized by the X-ray film were cut out and counted by liquid scintillation according to published methods.

RESULTS

E. coli strain W3350, which showed a sensitivity to bicyclomycin with an I_{50} value of between 25 and 30 μg/mL, was mutagenized by UV irradiation under conditions producing approximately 10% survival. The treated cells were spread on LB plates containing 200 μg/mL bicyclomycin. Twenty mutants, each showing varying degrees of bicyclomycin resistance, were isolated. The I_{50} values were determined, and a select set are plotted in Figure 2. A resistant mutant, BCMr108, with an I_{50} value of approximately 500 μg/mL, was chosen for the isolation of genomic DNA. This DNA was used to construct a partial *Sau*3AI-generated plasmid library ranging in size from 2 to 6 kbp, which was ligated into the *Bam*HI-linearized plasmid pBGS18 and transformed into *E. coli* strain ER1451.

Five bicyclomycin-resistant colonies arising from the recombinant DNA were replated on both bicyclomycin and kanamycin plates and grown on liquid media containing either bicyclomycin or kanamycin. The resulting plasmids pAZbcmr108-1 through pAZbcmr108-5 were restriction-digested

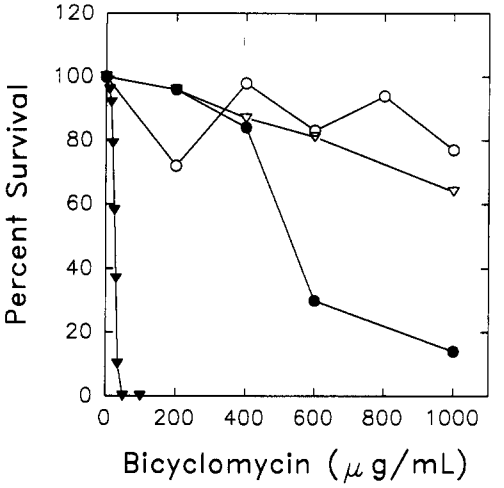

FIGURE 2: Survival curves for *E. coli* strains and mutants grown in the presence of varying concentrations of bicyclomycin: W3350 (▲); BCMr108 (●); BCMr110 (△); and BCMr107 (○).

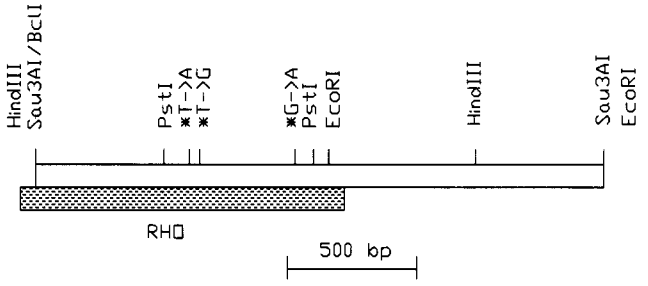

FIGURE 3: Restriction map of the 2.2-kbp partial *Sau*3AI digest restriction fragment contained in pAZbcmr108-2. The dashed bar indicates the position of the transcription termination factor rho gene. The asterisks indicate the positions of the three different mutations.

with *Hind*III, *Eco*RI, and *Hind*III plus *Eco*RI. The resulting restriction map of pAZbcmr108-2 is shown in Figure 3. All five isolated plasmid DNAs appeared to contain the same sized fragments. Plasmid DNA from pAZbcmr108-2 was reisolated and retransformed into ER1451, which again yielded bicyclomycin resistance cells carrying a plasmid with an identical size restriction fragment. Thus, we concluded that the cloned DNA conferred bicyclomycin resistance to the transformed cells. *Hind*III, *Eco*RI, and *Hind*III + *Eco*RI restriction fragments (see Figure 3) of the partial *Sau*3AI fragment from pAZbcmr108-2 were ligated into the proper pBGS18-linearized plasmids and transformed into ER1451. Of these subclones, the 1.7-kbp *Hind*III fragment and the 1.1-kbp *Hind*III–*Eco*RI showed bicyclomycin resistance, while the 1.1-kbp *Eco*RI fragment showed no bicyclomycin resistance (Figure 4). Subfragments from pAZbcmr108-2 were subcloned into M13mp18/19, and the sequence was determined by dideoxy chain termination and found to be encoding the transcription termination factor rho (Brown et al., 1982; Pinkham & Platt, 1983) by sequence comparison (Figure 5). The relative position of the rho factor gene within the cloned DNA in pAZbcmr108-2 is shown in Figure 3.

Examination of the DNA sequence of pAZbcmr108-2 with the previously determined sequence for rho indicated that a single base change at nucleotide position 1009, a G to A transition changing amino acid 337 from Gly to Ser, was responsible for the bicyclomycin resistance (Figure 5). Confirmation of this mutation was provided by sequencing wild-type W3350 around nucleotide position 1009 and determining that the sequence was identical to that reported for rho (Figure 6) (Pinkham & Platt, 1983). Upon closer

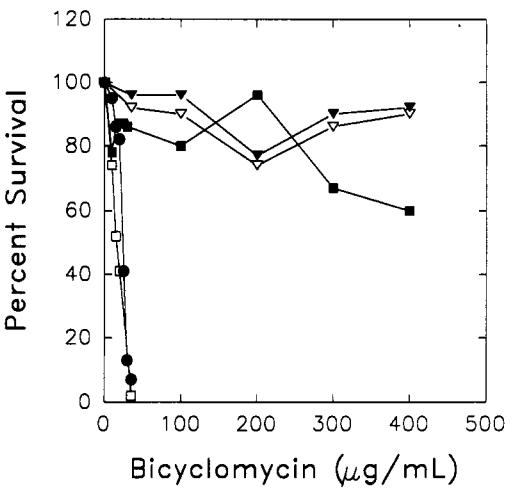

FIGURE 4: Survival curves for *E. coli* strains containing plasmids possessing mutations in the presence of varying concentrations of bicyclomycin: plasmid host, ER1451, with plasmid pBGS18 without an insert (●); ER1451 containing plasmid pAZbcmr108-2 (▲); ER1451 with pBGS18 containing the 1.7-kbp *Hind*III fragment (■); ER1451 with pBGS18 containing the 1.1-kbp *Hind*III–*Eco*RI fragment (△); and ER1451 with pBGS18 containing the 1.1-kbp *Eco*RI fragment (□).

examination of the *Sau*3AI partial fragment, we noted that the 41 nucleotides into the gene from the presumed start of translation of the rho gene were absent from the cloned fragment (Pinkham & Platt, 1983). Similarly, we found that the cloned 1.1-kbp *Hind*III–*Eco*RI fragment conferred bicyclomycin resistance, despite the fact that it lacks 19 amino acid codons from the 3′-end of the DNA. These observations indicated that mutant rho expression from the plasmid could not explain these results. Previous studies have reported that the Leu(3) residue of rho was shown to be important in transcription termination (Mori et al., 1989). This finding suggested that homologous recombination of the plasmid-bearing rho DNA into the host DNA may have been responsible for the bicyclomycin-resistant phenotype.

Additional information concerning the site of bicyclomycin inhibition was provided by the other UV-generated mutants. Sequence analysis of the 572-bp *Pst*I fragment from base 562 to 1134 of PCR-amplified DNA from the remaining 19 mutants showed that 16 of these possessed the original G to A transition at position 1009 found for pAZbcmr108-2, while two mutants BCMr107 and BCMr102 showed a base change of T to A at base position 656, changing amino acid residue at 219 from Met to Lys, and mutant BCMr110 contained a T to G transition at base position 796, resulting in the conversion of amino acid 266 from Ser to Ala. Amplified DNA from the two new mutants exhibiting the 1.1-kbp *Bcl*I–*Eco*RI fragment was ligated into pBGS18, transformed into ER1451, and tested for bicyclomycin resistance. The transformed cells containing the mutant pBGS18 clones were resistant to bicyclomycin at drug concentrations of 200 μg/mL, thereby reinforcing our finding that rho is the primary site of bicyclomycin action.

In order to support the molecular biological data of the site of bicyclomycin function, the biochemical effects of bicyclomycin on polyC-stimulated rho ATPase activity were examined. rho was purified from the overproducing strain AR120 containing the expression vector p39-AS carrying the rho gene. The protein was isolated according to the published methods up to and including the Bio-Rex-70 column chromatography. Peaks from the Bio-Rex column were pooled and assayed first on SDS–PAGE electrophoresis and then for polyC-stimulated

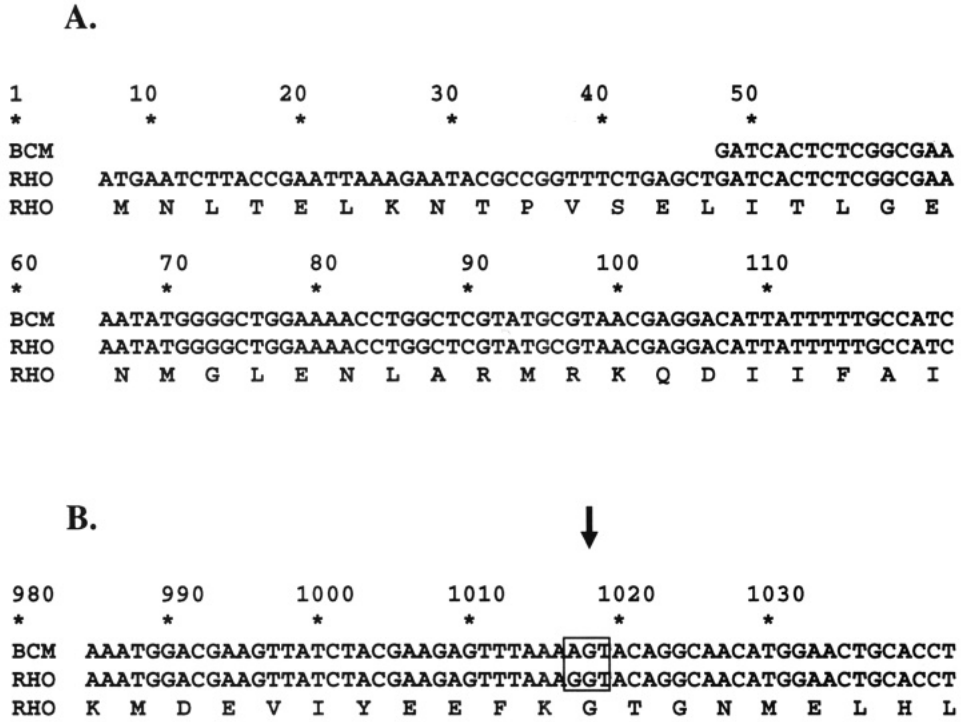

FIGURE 5: DNA sequences and the predicted protein sequence for the partial *Sau*3AI digest DNA fragment cloned in plasmid pAZbcmr108-2 and for the transcription termination factor rho: (A) the first 114 nucleotide residues of the coding sequence of rho and the beginning of the DNA sequence ligated in pAZbcmr108-2 encoding bicyclomycin resistance; (B) comparison of the wild-type and the mutant BCMr108 rho DNA sequences from residue 977 to 1034. The box and arrow indicate the codon changing from GGT (Gly) to AGT (Ser) at position 1009.

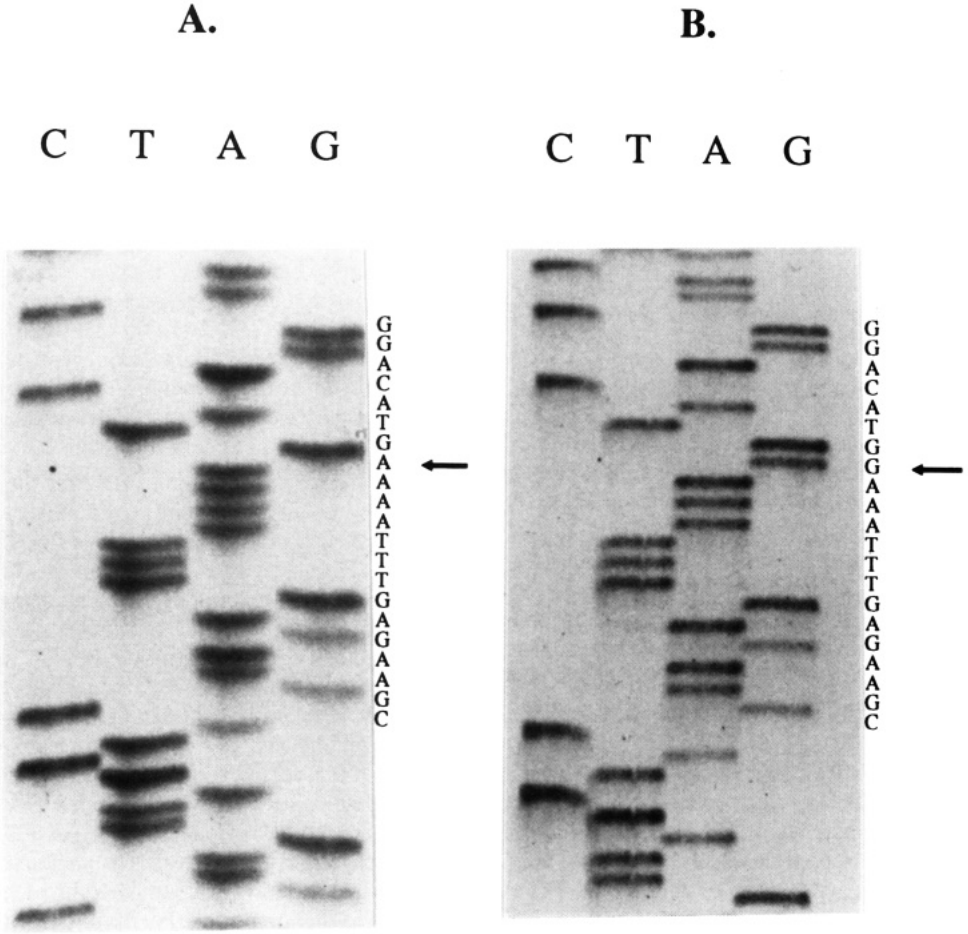

FIGURE 6: DNA sequencing gels indicating the mutant base change: (A) BCMr108; (B) W3340.

ATPase activity (Das et al., 1978). The release of terminal phosphate from γ-labeled ATP was determined by extraction in the presence of silicotungstate and molybdate and measured using a Beckman LS6000 liquid scintillation counter. Figure 7 shows the increase in the release of polyC-stimulated γ-^{32}P from ATP with time using two concentrations of enzyme.

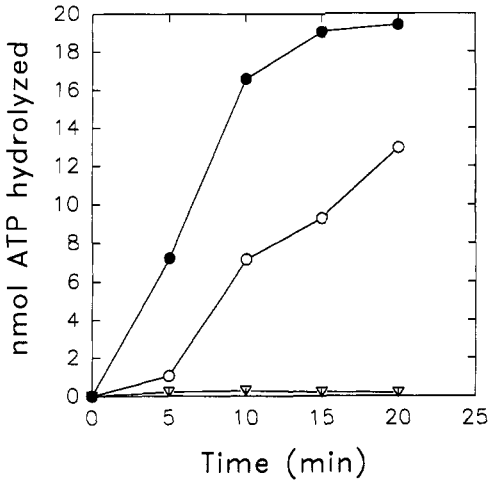

FIGURE 7: PolyC-stimulated rho ATPase activity in the absence (○, ●) and presence (△) of 66 μM bicyclomycin with time. The assay was carried out with 0.4 mM ATP, 1 μg of polyC, and 50 ng of rho (○) or 100 ng of rho (●). The values represent averages of two experiments.

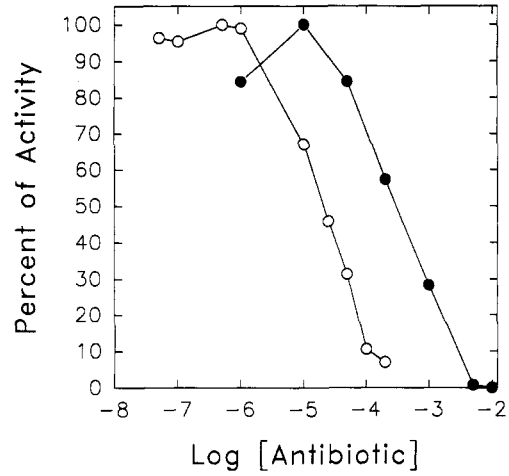

FIGURE 8: Inhibition of polyC-stimulated rho ATPase activity with varying concentrations (expressed as log of the molar concentrations) of bicyclomycin (○) and dihydrobicyclomycin (●). The assay was carried out for 10 min in the presence of 100 ng of rho, 0.4 mM ATP, 1 μg of polyC, and 500 nCi of γ-labeled ATP.

The maximal rate of enzymatic activity was 14.1×10^{-9} mol of ATP hydrolyzed per microgram of rho per minute at 32 °C. This value was comparable to the published rates of polyC-stimulated rho ATPase activity (Sharp et al., 1983). The addition of 66 μM bicyclomycin to the assay caused a decrease in the release of phosphate. Figure 8 shows the titration of rho activity with increasing bicyclomycin and dihydrobicyclomycin concentrations. Bicyclomycin possessed an I_{50} value of 60–70 μM on the ATPase activity of rho, while dihydrobicyclomycin showed a much less inhibitory effect with an I_{50} of 200–400 μM.

Mutant rho was isolated from BCMr108 and was characterized by mobility on SDS gel electrophoresis and by the polyC-stimulated ATPase activity. SDS–PAGE indicated the presence of a band of comparable relative molecular size versus wild-type rho, along with other protein bands. The effect of bicyclomycin on the ATPase activity of mutant rho from BCMr108 was evaluated. Figure 9 shows the time course for the catalyzed hydrolysis of ATP for wild-type and mutant rho in the presence and absence of 200 μM bicyclomycin. At this drug concentration, wild-type rho activity (Sharp et al., 1983) was completely abolished, while the fraction containing the mutant rho was unaffected. The titration curves for wild-type and mutant rho activity as a function of bicyclomycin concentration are provided in Figure 10. At 66 μM bicyclomycin, the mutant rho was completely active. This value corresponded to the I_{50} value for wild-type rho. Further increase in the bicyclomycin concentration by 150-fold to 10 mM led to only 40% inhibition of polyC-dependent ATPase activity.

DISCUSSION

Our investigations have led to the discovery that bicyclomycin resistance is encoded on a fragment of DNA carrying the coding region of the transcription termination factor rho. The essential nature of the rho factor in Gram-negative bacterial processes has been previously demonstrated by using temperature-sensitive (ts) *E. coli* mutants in rho, which failed to grow at elevated temperatures of 42 °C (Das et al., 1976; Inoko et al., 1977; Richardson & Carey, 1982), and by introduction of an inactivated plasmid copy of rho into the genome (Russel & Model, 1984). A general working mechanism for rho termination has been presented (Platt, 1986). rho binds to nascent RNA at *rut* sites, with termination occurring just downstream from this locus (Richardson, 1991). In a dynamic process in which ATP hydrolysis occurs, the rho factor translocates toward the 3′-end of the RNA. The termination step is the displacement of the RNA polymerase from the DNA template, causing the cessation of RNA synthesis. The rho factor has been divided into various binding domains, including the RNA binding segment and the ATP hydrolysis domain (Dombroski & Platt, 1990; Mori et al., 1989; Richardson, 1990).

Our finding that the plasmid-encoded BCMr rho mutants containing a shortened rho sequence expressed bicyclomycin resistance was unexpected. The initial lag times seen in the growth curves of the cells containing these plasmids when placed on bicyclomycin suggested that the mutant rho is not constituative but must be "induced" (data not shown). This lag time was not seen if the cells were already grown on bicyclomycin. This delay in bacterial growth could be explained by a recombination event producing a stable bicyclomycin-resistant loci in the genome of the host arising from the plasmid DNA, such that when challenged with the antibiotic, only the recombined cells survived.

Preliminary information concerning the mechanism of rho inhibition by bicyclomycin at the molecular level has been obtained. Our studies have demonstrated that polyC-dependent ATPase activity of rho was markedly diminished in the presence of 66 μM bicyclomycin. This decrease in biochemical activity closely resembled the concentration dependence on viable colony counts. Significantly, all three mutation sites (residues 219, 266, and 337) in the bicyclomycin-resistant mutants were found in the C-terminal half of the protein nearest to the putative ATP binding domain (Richardson, 1990; Dumbroski & Platt, 1990; Mori et al., 1989). This region also contains the only cysteine residue (position 202) found in rho. Previous studies have demonstrated that bicyclomycin can bond to functionalized cysteines at near-neutral pH values (Abuzar & Kohn, 1990). This finding leads us to the suggestion that bicyclomycin inhibition of rho-dependent processes stems from the binding of the antibiotic at or near the ATP binding domain first, followed by covalent bonding of this cysteine to the terminal double bond in bicyclomycin. In support of this notion, no loss of ATPase activity was observed with the same concentrations (66 μM)

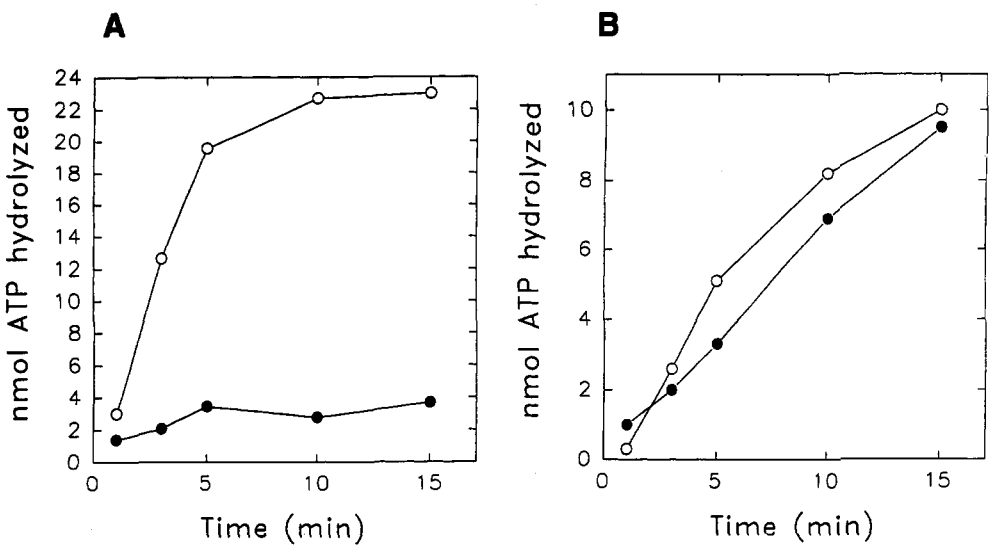

FIGURE 9: (A) Time course for the hydrolysis of 0.25 mM ATP in the absence (○) and presence (●) of 200 μM bicyclomycin for wild-type using 8 ng of rho. (B) Time course for the hydrolysis of 0.25 mM ATP in the absence (○) and presence (●) of 200 μM bicyclomycin for the partially purified mutant rho. The values represent the averages of two experiments.

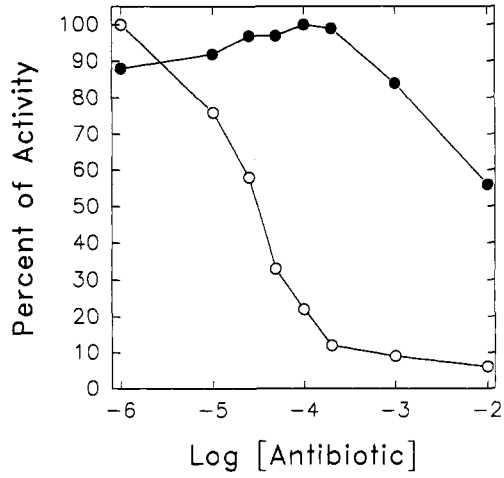

FIGURE 10: Inhibition of polyC-stimulated ATPase activity of wild-type (○) and mutant (●) rho with varying concentrations of bicyclomycin (expressed as log of the molar concentrations). The values represent averages of two measurements.

of dihydrobicyclomycin, a derivative of the antibiotic in which the *exo*-methylene group has been reduced. Moreover, at higher concentrations of dihydrobicyclomycin, inhibition of rho ATPase activity was seen, intimating that the structural aspect of binding to the ATP site on rho still exists in the dihydro derivative, but the cysteine bonding was lost. The observation that ATPase activity for the rho isolated from the mutant BCMr108 was only partially inhibited by bicyclomycin concentrations 150 times higher than that required for inhibition of wild-type activity provided additional evidence that rho is the site of action of bicyclomycin inhibition. This mode of inhibition is still under investigation, and further kinetic experiments may help resolve this issue.

Finally, we note that in polarity suppressor mutants used to study rho function (Das et al., 1978), the incorporation of [^{3}H]CTP into RNA increased over that of the wild type under restrictive temperatures (Das et al., 1976). This elevated RNA synthesis is similar to the increased levels of radioactivity incorporated into RNA previously seen in the presence of bicyclomycin (Someya et al., 1978). Currently, experiments are underway to determine the site and mechanism of drug action and to characterize the plasmid-transformed mutants for homologous recombination.

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REFERENCES

Abuzar, S., & Kohn, H. (1990) *J. Am. Chem. Soc. 112*, 3114–3121.

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., & Struhl, K. (1989) in *Current Protocols in Molecular Biology*, Greene Publishers and Wiley Interscience, New York.

Brown, S., Albrechtsen, B., Pedersen, S., & Klemm, P. (1982) *J. Mol. Biol. 162*, 283–298.

Das, A., Court, D., & Adhya, S. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1959–1963.

Das, A., Merril, C., & Adhya, S. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4828–4832.

Devereux, J., Haeberli, P., & Smithies, O. (1984) *Nucleic Acids Res. 12*, 387–395.

Dillela, A. G., & Woo, S. L. C. (1985) *Focus 7*, 1–5.

Dombroski, A. J., & Platt, T. (1990) *J. Bacteriol. 172*, 2477–2484.

Finger, L. R., & Richardson, J. P. (1981) *Biochemistry 20*, 1640–1645.

Inoko, H., Shigesdà, K., & Imai, M. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 1163–1166.

Kamiya, T., Maeno, S., Hashimoto, M., & Mine, Y. (1972) *J. Antibiot. 25*, 576–581.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Martin, H. H., Lehmann, R., Herzog, U., & Kaul, U. (1974) *Ann. N.Y. Acad. Sci. 235*, 283–293.

Miyoshi, T., Miyari, N., Aoki, H., Koshaska, M., Sakai, H., & Imanaka, H. (1972) *J. Antibiot. 25*, 569–576.

Mori, H., Imai, M., & Shigesada, K. (1989) *J. Mol. Biol. 210*, 39–49.

Myamura, S., Ogasawara, N., & Otsuka, H. (1972) *J. Antibiot. 25*, 610–612.

Pinkham, J. L., & Platt, T. (1983) *Nucleic Acids Res. 11*, 3531–3545.

Pisabarro, A. G., Canada, F. J., Vasquez, D., Arriaga, P., & Rodriguez-Tebar, A. (1986) *J. Antibiot. 39*, 914–921.

Platt, T. (1986) *Annu. Rev. Biochem. 55*, 339–372.
Richardson, J. P. (1990) *Biochim. Biophys. Acta 1048*, 127–138.
Richardson, J. P. (1991) *Cell 64*, 1047–1049.
Richardson, J. P., & Carey, J. L., III (1982) *J. Biol. Chem. 257*, 5767–5771.
Russel, M., & Model, P. (1984) *J. Bacteriol. 159*, 1034–1039.
Saiki, R. K., Scarf, S., Faloona, F., Mulliks, K. B., Hron, G. T., Erlich, H. A., & Arnheim, N. (1985) *Science 230*, 1350–1354.
Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463–5467.
Sharp, J. A., Galloway, J. L., & Platt, T. (1983) *J. Biol. Chem. 258*, 3482–3486.
Someya, A., Iseki, M., & Tanaka, N. (1978) *J. Antibiot. 31*, 712–718.
Stryer, L. (1988) *Biochemistry*, 3rd ed. pp 714–715, W. H. Freeman, New York.
Yager, T. D., & von Hippel, P. H. (1987) in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology* (Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schechter, M., & Umbarger, H. E., Eds.) pp 1241–1275, American Society of Microbiology, Washington, DC.