SELF-RESISTANCE IN ACTINOMYCETES PRODUCING INHIBITORS OF RNA POLYMERASE

J. ROZA, M. G. BLANCO, C. HARDISSON and J. A. SALAS*

Departamento de Microbiologia,
Universidad de Oviedo,
33006 Oviedo, Spain

(Received for publication November 5, 1985)

A number of antibiotics produced by different actinomycete species inhibit DNA-dependent RNA polymerase. These include the ansamycin group (rifamycins, streptovaricins, tolypomycins and geldanamycin) and streptolydigin, tirandamycin, and thiolutin. The producing organisms of these antibiotics must possess resistance mechanisms to overcome the toxic effects of the antibiotics. Target site modification and the consequent inability of the antibiotic to bind to its site of action is a widespread resistance mechanism among antibiotic-producing organisms[1]. It has been shown that the *in vitro* RNA polymerase activity of *Streptomyces* (now *Nocardia*) *mediterranei* was resistant to the antibiotic produced (rifamycin)[2]. In addition, we have shown[3] that modification of the antibiotic target site is responsible for the resistance of *Streptomyces spectabilis* (streptovaricin producer) and *Streptomyces lydicus* (streptolydigin producer) to their own products. We have now extended these studies to other organisms producing RNA polymerase inhibitors, to test whether the existence of a resistant RNA polymerase can be considered a general mechanism in such producing organisms. Furthermore, we were interested in studying cross-resistance among these antibiotics.

Microorganisms and Culture Conditions

The actinomycetes used in this study were: *Nocardia mediterranei* ATCC 13685, *S. spectabilis* UC 2472, *S. lydicus* UC 2056, *S. hygroscopicus* var. *geldanus* UC 5208 and *S. tolypophorus* NRRL B-12585. The organisms were grown in YEME liquid medium (g/liter): glucose 10.0, peptone 5.0, yeast extract 3.0, malt extract 3.0. After 36 hours of incubation at 35°C with shaking, the mycelia were harvested by centrifugation and washed twice with 10 mM Tris-HCl, pH 8.0, containing 1 mM EDTA, 10 mM $MgCl_2$, 50 mM KCl, 7 mM 2-mercaptoethanol, 5% glycerol and 1 mM phenylmethylsulfonyl fluoride.

Determination of Minimal Inhibitory Drug Concentrations

Conditions were as described previously[3].

Partial Purification of RNA Polymerase

The mycelia were broken by ultrasound and, after centrifugation of $100,000 \times g$ for 2 hours, the supernatant was submitted to ammonium sulfate fractionation at 35% and 70% saturation. The latter precipitate was dissolved in, and dialyzed extensively against the buffer mentioned above and then applied to a heparin-agarose column. After washing the column with the buffer, it was eluted with KCl (linear concentration gradient 0.05 M to 0.65 M).

Assay of RNA Polymerase Activity

RNA polymerase activity of the five organisms tested was assayed using a synthetic DNA (poly dA-dT) as template. The assay mixture contained, in a total volume of 100 μl, the following components: 50 mM Tris-HCl, pH 7.9, at 25°C, 10 mM $MgCl_2$, 10 mM $MnCl_2$, 200 mM KCl, 0.1 mM dithiothreitol, 0.1 mM EDTA, 1 mM K_2HPO_4, 0.1 mM GTP, 0.1 mM CTP, 0.1 mM UTP, 0.05 mM [2,5,8-^{3}H]ATP (10 μCi/ml; 46 Ci/mmol; Amersham International), 0.05 μg bovine serum albumin, 1 μg poly dA-dT and 5~50 μl extract. The reaction was stopped after 15 minutes at 37°C by the addition of 1 ml 5% (w/v) trichloroacetic acid (TCA) containing 10 mM sodium pyrophosphate. After 30 minutes at 4°C, the samples were filtered through Whatman GF/C glass fiber discs and washed with 20 ml 2% TCA plus 10 mM sodium pyrophosphate. The radioactivity in the dried filters was estimated in a liquid scintillation spectrophotometer.

All the producers of RNA polymerase inhibitors tested were resistant *in vivo* to the antibiotics they produce (Table 1). Cross-resistance was found only in the case of *S. tolypophorus* (tolypomycin producer) which was resistant to all the ansamycins (streptovaricin, rifamycin, geldanamycin and tolypomycin) and for *N. mediterranei* which was resistant to rifamycin and tolypomycin. Thiolutin was a strong inhibitor of most of the organisms.

Table 1. Minimal inhibitory concentrations of antibiotic producer.

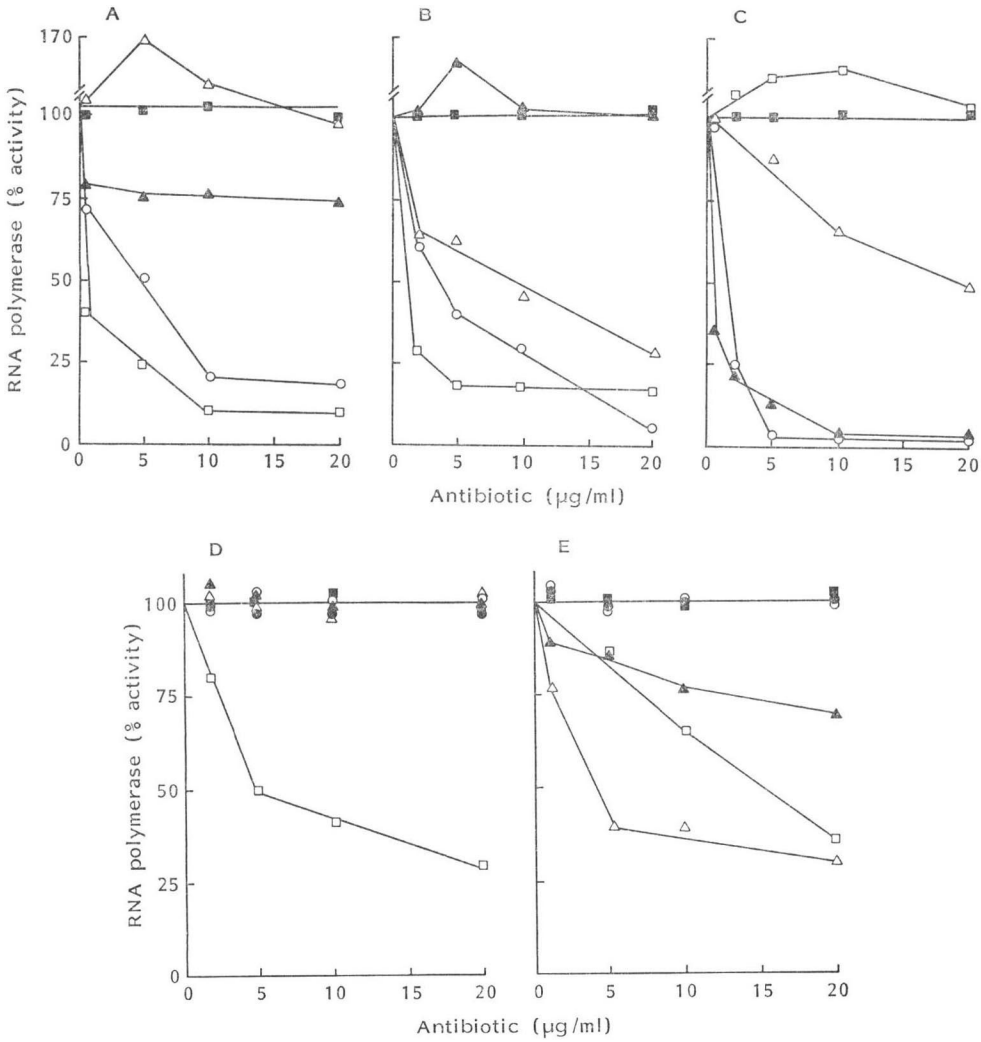
Organism	Produced antibiotic	MIC (μg/ml)					
		TOL	GEL	RIF	STV	SLG	THIO
Streptomyces tolypophorus	TOL	>200	>200	>200	>200	5	25
S. hygroscopicus var. *geldanus*	GEL	75	>200	75	100	25	<1
Nocardia mediterranei	RIF	>200	25	>200	100	10	<1
S. spectabilis	STV	25	75	100	>200	10	<1
S. lydicus	SLG	50	25	25	25	>200	25

Abbreviations: TOL; Tolypomycin Y, GEL; geldanamycin, RIF; rifamycin SV, STV; streptovaricin, SLG; streptolydigin, THIO; thiolutin.

Fig. 1. Effect of antibiotics on *in vitro* RNA polymerase activity.

RNA polymerase activity of each producer was assayed in the presence of different antibiotic concentrations using poly dA-dT as template.

A; *Streptomyces hygroscopicus*, B; *S. spectabilis*, C; *S. lydicus*, D; *S. tolypophorus*, E; *Nocardia mediterranei*, ○ rifamycin SV, ● tolypomycin Y, △ geldanamycin, ▲ streptovaricin, □ streptolydigin, ■ thiolutin.



In preliminary experiments, incubation with cell-free extracts from the five antibiotic-producing strains failed to inactivate any of the RNA polymerase inhibitors, even when supplemented with acetyl CoA and ATP (data not given); therefore, the producers did not appear to contain antibiotic-inactivating enzymes. However, the RNA polymerase activity was found to be resistant to the endogenous antibiotic (Fig. 1 A~E) and in some cases (*e.g. S. hygroscopicus*, *S. spectabilis* and *S. lydicus*) was even stimulated at low concentrations. Complex patterns of cross-resistance among the antibiotics were evident at the level of RNA polymerase. Thus (Fig. 1 D), the enzyme from *S. tolypophorus* (tolypomycin producer) was completely resistant to all the assayed antibiotics of the ansamycin group (streptovaricin, geldanamycin, rifamycin and tolypomycin) whereas RNA polymerase activity from the three other ansamycin producers did not show such pattern of cross-resistance. *N. mediterranei* RNA polymerase was completely resistant to rifamycin and, partially, to streptovaricin while *S. hygroscopicus* RNA polymerase was resistant to geldanamycin and, partially, to streptovaricin. On the other hand, the other ansamycin producer, *S. spectabilis*, was specifically resistant to streptovaricin. It is noteworthy that *S. lydicus* RNA polymerase was resistant to streptolydigin but very sensitive to tirandamycin (data not shown), despite the close structural similarity between these two antibiotics and the fact that tirandamycin is 40 times less potent than streptolydigin[4].

Thiolutin is a sulfur-containing antibiotic[5] which has been shown to be an inhibitor of yeast RNA polymerases *in vitro*[6,7] and it has been suggested that prokaryotic RNA synthesis is also sensitive to this antibiotic[8,9]. However, we found that the RNA polymerases of the five organisms used in this study were unaffected by thiolutin *in vitro* (Fig. 1 A~E), although they were very sensitive to the drug *in vivo* (see Table 1). We therefore suggest that, at least in several actinomycetes, thiolutin is not an inhibitor of *in vitro* RNA polymerase activity using poly dA-dT as a synthetic template and that its inhibitory action against such prokaryotes remains obscure.

In summary, resistance at the level of the antibiotic target site may be a mechanism of self defence among actinomycetes producing inhibitors of RNA polymerase. Cross resistance among these antibiotics is not unusual[10~12], however drug-tolerance among the producers seems to be more selective. It is possible that during evolution the producing organisms have developed specific modifications of RNA polymerase, either in the amino acid sequence or by enzymatic modification. The molecular mechanisms responsible for such resistance are being studied.

Acknowledgments

The authors wish to thank Dr. ERIC CUNDLIFFE for a critical reading of the manuscript. We also wish to thank Upjohn Co. for the gift of streptovaricin, streptolydigin, tirandamycin and geldanamycin, Takeda Chemical Industries for tolypomycin Y and Pfizer for thiolutin. This research was supported by a grant of the Comisión Asesora para el desarrollo de la Investigación Cientifico-Técnica, Spain (CAICYT No. 2107/83) and by a Acción Integrada Hispano-Británica (No. 18/23).

References

1) CUNDLIFFE, E.: Self-defence in antibiotic-producing organisms. Br. Med. Bull. 40: 61~67, 1984
2) WATANABE, S. & K. TANAKA: The effect of rifampicin on *in vitro* RNA synthesis of *Streptomyces mediterranei*. Biochem. Biophys. Res. Commun. 72: 522~529, 1976
3) BLANCO, M. G.; C. HARDISSON & J. A. SALAS: Resistance to inhibitors of RNA polymerase in actinomycetes which produce them. J. Gen. Microbiol. 130: 2883~2891, 1984
4) REUSSER, F.: Tirandamycin, an inhibitor of bacterial ribonucleic acid polymerase. Antimicrob. Agents Chemother. 10: 618~622, 1976
5) CELMER, W. D. & I. A. SOLOMON: The structures of thiolutin and aureothricin, antibiotics containing a unique pyrrolinodithiol nucleus. J. Am. Chem. Soc. 77: 2861~2865, 1955
6) JIMENEZ, A.; D. J. TIPPER & J. DAVIES: Mode of action of thiolutin, an inhibitor of macromolecular synthesis in *Saccharomyces cerevisiae*. Antimicrob. Agents Chemother. 3: 729~738, 1973
7) TIPPER, D. J.: Inhibition of yeast ribonucleic acid polymerases by thiolutin. J. Bacteriol. 116: 245~256, 1973
8) KHACHATOURIANS, G. G. & D. J. TIPPER: Inhibition of messenger ribonucleic acid synthesis in *Escherichia coli* by thiolutin. J. Bacteriol. 119: 795~804, 1974
9) SIVASUBRAMANIAN, N. & R. JAYARAMAN: Thi-

olutin resistant mutants of *Escherichia coli*: Are they RNA chain initiation mutants? Mol. Gen. Genet. 145: 89~96, 1976

10) YURA, T. & K. IGARASHI: RNA polymerase mutants of *Escherichia coli*. I. Mutants resistant to streptovaricin. Proc. Natl. Acad. Sci. U.S.A. 61: 1313~1319, 1968

11) SONENSHEIN, A. L.; B. CAMI, J. BREVET & R. COTE: Isolation and characterization of rifampicin-resistant and streptolydigin-resistant mutants of *Bacillus subtilis* with altered sporulation properties. J. Bacteriol. 120: 253~265, 1974

12) MORROW, T. O. & S. A. HARMON: Genetic analysis of *Staphylococcus aureus* RNA polymerase mutants. J. Bacteriol. 137: 374~383, 1979