LIPIARMYCIN: AN ANTIBIOTIC INHIBITING NUCLEIC ACID POLYMERASES+

M. Talpaert, F. Campagnari and L. Clerici

Biology Group, Ispra
G.D. Research, Science and Education, C.E.C.
EURATOM J.R.C., 21020 Ispra (Italia)

Received January 16, 1975

SUMMARY

Lipiarmycin, a new antibiotic from a strain of Actinoplanes, was found to inhibit RNA polymerase from E. coli by blocking the initiation step of RNA synthesis like rifampicin. The drug was a much less potent and specific inhibitor than rifampicin: doses 10 to 20 times higher than those effective with the E. coli enzyme antagonized the mammalian RNA polymerases A and B as well as the DNA polymerases from prokaryotic and from eukaryotic cells. Both rifampicin and lipiarmycin acted as non-competitive inhibitors of either UTP or ATP substrates in the synthesis of the respective homopolymers promoted by the bacterial RNA polymerase with $(dA)_n \cdot (dT)_n$ templates.

INTRODUCTION

Lipiarmycin is a new antibiotic isolated from a strain of Actinoplanes in the Lepetit Laboratories (Milan). It is a compound of low molecular weight (about 1100) with a chemical structure incompletely known. In bacteria, the antibiotic blocked the synthesis of RNA and impaired the replication of DNA to a smaller extent (1,2).

We analyzed the effects of lipiarmycin on the reactions promoted "in vitro" by the common RNA and DNA polymerases from E. coli and from calf thymus cells. Especially, the mechanism of the inhibition exerted by the antibiotic in the catalysis of the ribonucleotidyltransferases was studied. Here, the efforts were directed to clarify which step of RNA polymerization was primarily affected by the drug and which type of changes in the enzyme affinity for the nucleotide substrates were induced. For sake of comparison, the action of rifampicin on the RNA polymerase of E. coli was investigated using the same experimental techniques.

+ Contribution N° 975 of the Biology Programme, Directorate General XII of the Commission of the European Communities.

Copyright © 1975 by Academic Press, Inc.
All rights of reproduction in any form reserved.

MATERIALS AND METHODS

Biochemicals - Radioactive and nonradioactive nucleoside triphosphates were obtained from NEN and P.L. Biochemicals, Inc., respectively.

DNA and polydeoxynucleotides - Calf thymus DNA from Worthington was further purified and reisolated according to Kedinger et al.(3). $d(A)_n \cdot d(T)_n$ and $d(A)_n \cdot d(T)_{12}$ were routine preparations of our laboratory.

Enzymes - The RNA polymerase from E. coli was the enzyme isolated by the method of Berg et al.(4). RNA polymerases A and B were prepared from calf thymus nuclei as described by Chesterton and Butterworth (5,6). E. coli DNA polymerase I was the hydroxylapatite fraction obtained according to Richardson et al. (7). The nuclear DNA polymerase from calf thymus was purified according to Chang (8).

Enzyme assays

E. coli RNA polymerase. The reaction mixture contained: 50 mM Tris-HCl (pH 8.0) ; 10 mM $MgCl_2$; 100 mM KCl; 20 mM mercaptoethanol; 200 μg of calf thymus DNA per ml; 0.34 mM CTP and GTP; 0.46 mM ATP and [^{14}C]UTP labeled with 0.2 μCi per ml.

RNA polymerases from calf thymus . The reaction mixture contained: 3 mM $MgCl_2$; 2 mM $MnCl_2$; 0.67 mM CTP and GTP; 0.93 mM ATP and 0.20 mM [^{14}C]UTP labeled with 0.2 μCi per ml; buffer, mercaptoethanol, and DNA as in the preceding mixture.

E. coli DNA polymerase. The reaction mixture was practically that proposed by Richardson (7) with a 50% increase of the total concentration of dNTP substrates and with the substitution of 0.1 μCi of [^{14}C]dTTP for the [α-^{32}P] dATP.

3.39 S nuclear DNA polymerase from calf thymus. The enzyme assay was carried out as previously reported (9).

The enzymatic assays of nucleotide polymerization were monitored by measuring the incorporation of radioactivity into acid-insoluble material according to the disk method previously described (10). One unit of DNA or RNA polymerase was defined as the amount catalyzing the incorporation of one nanomole of radioactively labeled nucleotides during 10 min incubation at 37° under the assay conditions.

RESULTS

Lypiarmycin was found to be a rather general inhibitor of the enzymes polymerizing the nucleic acids. As shown in Fig. 1 a, its activity was maximal against the RNA polymerase from E. coli and it was ten times lower with the corresponding mammalian ribonucleotidyltransferases. Five fold

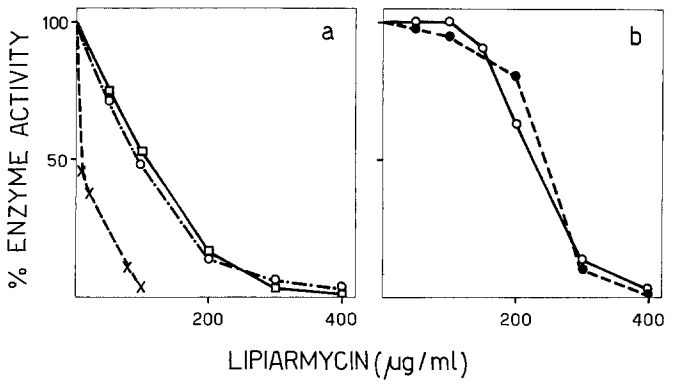

Fig. 1 a. Effect of lipiarmycin on RNA polymerases from E. coli and from calf thymus. Incubation was 10 min at 37° and reaction mixtures were of 1 ml. ● E. coli RNA polymerase (20 units); O calf thymus RNA polymerase A (5 units); □ calf thymus RNA polymerase B (6 units).

1 b. Effect of lipiarmycin on DNA polymerases from E. coli and from calf thymus. ● E. coli DNA polymerase (6 units); O calf thymus DNA polymerase (10 units). See Material and Methods.

---

increase or five fold decrease of the active proteins caused small shifts to the right or to the left, respectively, in the recorded curves of enzyme inhibition. No significant changes in the data were observed by varying the concentration of DNA or by preincubating the antibiotic and the enzyme with or without DNA. Very large doses of lipiarmycin were required to counteract the DNA polymerase from E. coli and from calf thymus cells (Fig. 1 b).

The effects of lipiarmycin on the kinetics of the reaction promoted by the bacterial ribonucleotidyltransferase were similar with those displayed by rifampicin, which remained a more powerful inhibitor in terms of the concentration necessary for eliciting a given response. As shown in Fig. 2, the initial presence of suitable amounts of either lipiarmycin or rifampicin in the mixtures for assaying the enzyme totally prevented the formation of RNA products. Otherwise, the addition of the antibiotics in the course of the nucleotide polymerization did not block immediately the reaction, but it gradually induced its levelling off. This is consistent with the view that lipiarmycin interfered with the initiation rather than with the elongation step of RNA synthesis. Thus, the antibiotic prevented the starting of new RNA chains, but it allowed the completion of those whose assembling had already begun. The less relevant inhibitions exerted by lipiarmycin on the mammalian RNA polymerases A and B seemed to occur by the same mechanism observed with the bacterial enzyme (Fig. 3).

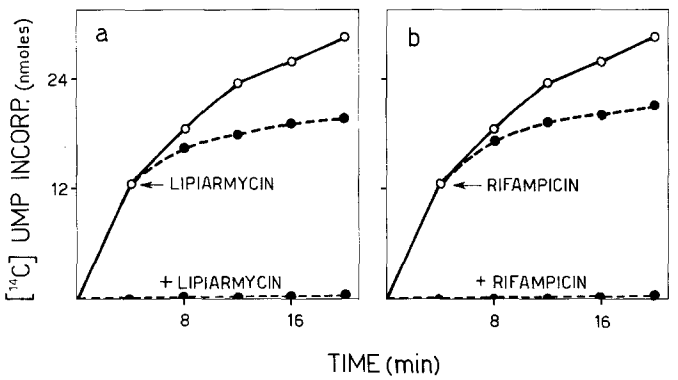

Fig. 2 a. Effect of lipiarmycin on the kinetics of the reaction catalyzed by E. coli RNA polymerase. The incubation mixtures were of 1 ml and contained 20 units of enzyme; 200 µg of lipiarmycin were added at the indicated time.

2 b. Effect of rifampicin on the kinetics of the reaction catalyzed by E. coli RNA polymerase. The experimental conditions were as in a, but 20 µg of rifampicin substituded for lipiarmycin. See Material and Methods.

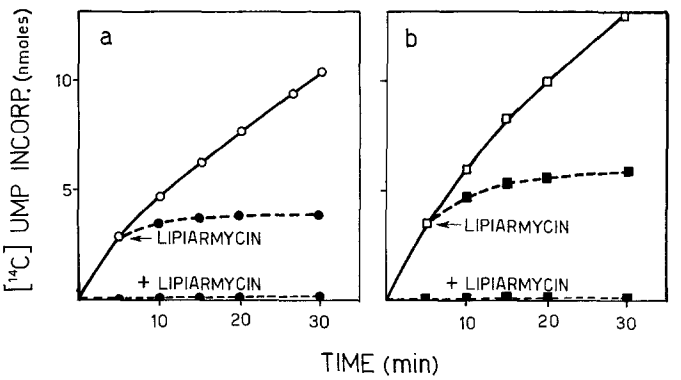

Fig. 3.a. Effect of lipiarmycin on the kinetics of the reaction catalyzed by RNA polymerase A. The incubation mixtures were of 1 ml and contained 5 units of enzyme; 400 µg of antibiotic were added at the indicated time.

3 b. Effect of lipiarmycin on the kinetics of the reaction catalyzed by RNA polymerase B. The experimental conditions were the same as in a, with the substitution of 6 units of enzyme B for the RNA polymerase A. See Material and Methods.

Using $(dA)_n \cdot (dT)_n$ as a template and the proper nucleoside triphosphates as substrates, it was possible to study separately the effect of rifampicin and lipiarmycin on the polymerizations of purine and pyrimidine nucleotides. Under the given experimental conditions, the apparent Km's of the RNA polymerase for the ATP and for the UTP substrates were practical-

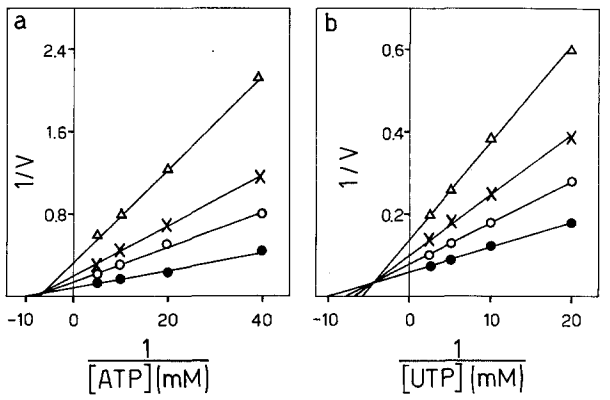

Fig. 4 a. Reciprocal plot of RNA polymerase activity and of ATP concentrations with and without lipiarmycin. The reaction mixtures contained: 50 mM Tris-HCl (pH 8.0); 8 mM $MgCl_2$; 20 mM mercaptoethanol; 2.5 mM $(dA)_n.(dT)_n$ as total nucleotide phosphorus; 5 units of E. coli enzyme per ml and increasing concentrations of [^{14}C] ATP. The incubation was of 3 min at 37° and the rates of catalysis were expressed as nmoles of nucleotide polymerized per ml in 10 min. ●, no lipiarmycin; O, 10 µg of lipiarmycin per ml; X, 25 µg of lipiarmycin per ml; Δ, 50 µg of lipiarmycin per ml.

4 b. Reciprocal plot of RNA polymerase activity and of UTP concentrations with and without lipiarmycin. The reaction mixtures contained: buffer, mercaptoethanol, $(dA)_n.(dT)_n$ and enzyme as in a; 2 mM $MgCl_2$; 2mM $MnCl_2$ and increasing concentrations of [^{14}C] UTP. The incubation was of 3 min and the rates of catalysis were expressed as nmoles of nucleotide polymerized per ml in 10 min. ●, O, X, Δ as in a.

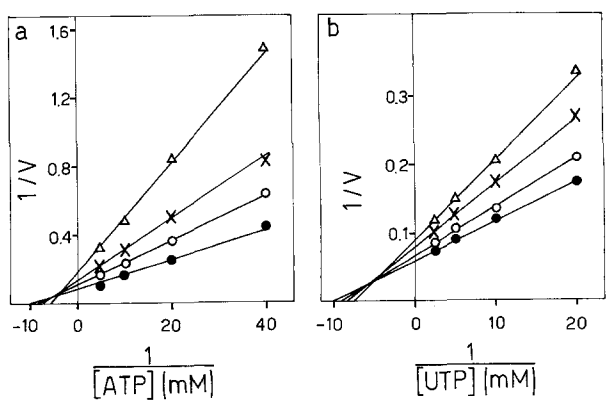

Fig. 5 a. Reciprocal plot of RNA polymerase activity and of ATP concentrations with and without rifampicin. The experimental conditions were the same as those described in the legend of Fig. 4 a with the substitution of rifampicin for lipiarmycin. ●, no rifampicin; O, 0.05 µg of rifampicin per ml; X, 0.10 µg of rifampicin per ml; Δ, 0.25 µg of rifampicin per ml.

5 b. Reciprocal plot of RNA polymerase activity and of UTP concentrations with and without rifampicin. The experimental conditions were the same as those described in the legend of Fig. 4 b with the substitution of rifampicin for lipiarmycin. ●, O, X, Δ as in a.

TABLE I

Inhibition of E. coli RNA polymerase by rifampicin and lipiarmycin. Apparent dissociation constants (Ki) of the enzyme-inhibitor complexes in the presence of $(dA)_n \cdot (dT)_n$ template and substrates for AMP or UMP polymerization.

	AMP polymerization	UMP polymerization
Ki for rifampicin-complex	1.3×10^{-7}M	3.2×10^{-7}M
Ki for lipiarmycin-complex	1.2×10^{-5}M	2.3×10^{-5}M

The experiments for determination of Ki's were conducted under the conditions described in the legends of Figs. 4 and 5. The Ki's were calculated by plotting $1/v$ versus the molar concentration of inhibitor, i, according to the method of Dixon (11).

ly identical and amounted to 10^{-4}M. As indicated by the inhibition patterns of the enzyme kinetics, lipiarmycin (Fig. 4) as well as rifampicin (Fig. 5) behaved as non competitive inhibitors of either ATP or UTP substrates. The two antibiotics displayed the same mechanism of action, although their ability to antagonize the bacterial enzyme was quite different. As indicated by the Lineweaver-Burk plots at different levels of inhibition (Figs. 4 and 5), lipiarmycin and rifampicin combined with the catalytic protein at sites which were partially or totally different from those specific for the ATP and the UTP substrates. However, the apparent dissociation constants Ki's determined by the method of Dixon (11) for the complexe between the RNA polymerase and lipiarmycin in the presence of ATP or UTP were 100 times higher than those observed with rifampicin under the given conditions for homopolymer condensation (Table I). From the same Table, it appears that the affinity of both antibiotics for the enzyme was twice as higher in polymerization system for ATP than in the one for UTP. This observation may suggest that lipiarmycin and rifampicin preferentially inhibited the reaction of purine nucleotides with the E. coli RNA polymerase.

DISCUSSION

It is worthy of consideration the fact that two antibiotics, much different in structure, such as lipiarmycin and rifampicin, were found to block the RNA polymerase reaction by a similar mechanism. Lipiarmycin is a less active and a more unspecific enzyme inhibitor and it would be interesting to determine what chemical structure or functional active

groups the two compounds have in common and how these similarities are related with the antibiotic properties. These informations may well be important in enhancing the potency of the lipiarmycin by proper chemical modifications.

Straat and coworkers were the first to introduce the use of $(dA)_n \cdot (dT)_n$ as a template for RNA polymerase studies concerning the enzyme from Micrococcus lysodeikticus (12) and from Micrococcus luteus (13). The double stranded homopolymer acted as a functional template yielding typical Michaelis-Menten kinetics with ATP or UTP substrates under optimal assay conditions. Our results are essentially in agreement with those findings and show that the RNA polymerase holoenzyme from E. coli was able to effect $(dA)_n \cdot (dT)_n$ directed polymerization of rU or rA nucleotides with high efficiency. This selective "in vitro" synthesis of purine or pyrimidine RNA chains by RNA polymerase may be exploited to study the interaction between inhibitors and catalytic sites for specific ribonucleotide substrates.

ACKNOWLEDGEMENTS

We are grateful to Dr. G.C. Lancini from Lepetit Laboratories for having provided the sample of lipiarmycin.

REFERENCES

1. Parenti, F., Pagani, H. and Beretta, A. submitted for publication in J. of Antibiotics.
2. Coronelli, L., White, R.J., Lancini, G.C. and Parenti, F. submitted for publication in J. of Antibiotics.
3. Kedinger, C., Gissinger, F., Gniazdowski, M., Mandel, J.-L. and Chambon, P. (1972) Eur. J. Biochem. 28, 269-276.
4. Berg, D., Barrett, K. and Chamberlin, M. (1971) Methods in Enzymology 21, 506-520.
5. Chesterton, C.J. and Butterworth, P.H.W. (1971) FEBS Letters 15, 181-185.
6. Chesterton, C.J. and Butterworth, P.H.W. (1971) FEBS Letters 12, 301-308.
7. Richardson, C.C., Schildkraut, C.L., Vasken Aposhian, H. and Kornberg, A. (1964) J. Biol. Chem. 239, 222-231.
8. Chang, L.M.S. (1973) J. Biol. Chem. 248, 3789-3795.
9. Bekkering-Kuylaars, S.A.M. and Campagnari, F. (1974) Biochim. Biophys. Acta 349, 277-295.
10. Campagnari,F., Bertazzoni, U. and Clerici, L. (1967) J. Biol. Chem. 242, 2168-2171.
11. Dixon, M. (1953) Biochem. J. 55, 170-177.
12. Straat, P.A., Ts'O, P.O.P. and Bollum, F.J. (1969) J. Biol. Chem. 244, 391-398.
13. Straat, P.A. and Ts'O,P.O.P. (1969) J. Biol. Chem. 244, 6263-6269.