MECHANISM OF ACTION OF A 16-MEMBERED MACROLIDE

CHARACTERISTICS OF DIHYDROROSARAMICIN BINDING TO *ESCHERICHIA COLI* RIBOSOME AND THE EFFECTS OF SOME COMPETITORS

SYLVIE SIEGRIST, SYLVIE VELITCHKOVITCH, FRANÇOIS LE GOFFIC and NICOLE MOREAU

CNRS-CERCOA 2 à 8, rue Henry Dunant, 94320 Thiais, France,
Laboratoire associé à l'ENSCP.

(Received for publication December 21, 1981)

The macrolide [^{3}H]dihydrorosaramicin binds specifically to 50S and 70S bacterial ribosomal particles. We have studied the influence of salts, pH and additives on the interaction and found that the optimum requirement for salts was 10 mM tris-HCl (pH 7.6), 6 mM $MgCl_2$, 60 mM NH_4Cl, and that β-mercaptoethanol which reacts on rosaramicin and its dihydro derivative cannot be used. The parameters of the binding were not dependent on the technique used, *i.e.* equilibrium dialysis, ethanol precipitation or two-phase partitioning. In our search for effectors of this binding, we have found that it is inhibited by other macrolides, little effected by tobramycin and chloramphenicol and enhanced by puromycin.

Rosaramicin is a member of the 16-membered macrolide family of antibiotics and displays fair antibacterial properties. We have shown in a previous paper[1] that its derivative [^{3}H]-20,20-dihydrorosaramicin binds reversibly and exclusively to the 70S or 50S ($K_D=0.2\times10^{-6}$ M and $K_D=0.16\times10^{-6}$ M, respectively) but not to the 30S ribosomal particle. This derivative is fully competitive with rosaramicin so it can be used to evaluate the binding of the parent compound. As only the standard buffer and technique (equilibrium dialysis) were used in this previous investigation, it is essential here to determine exact conditions for optimal binding. It is also of interest to see whether other antibiotics, well known for their affinity to the bacterial ribosome, will be able to modify the binding of [^{3}H]dihydrorosaramicin. Various antibiotics are assayed: other macrolides, tobramycin, chloramphenicol, puromycin, tetracycline.

Materials and Methods

Chemicals

Rosaramicin was a gift from Unilabo (France). [^{3}H]-20,20-Dihydrorosaramicin was synthesized in the laboratory[1]. Puromycin and tetracycline were provided by Serva. Chloramphenicol came from Sigma, erythromycin from Roussel-Uclaf, midecamycin (espinomycin A_1, 3-*O*-propionyl analogue of leucomycin A_6) from Clin-Midy, tobramycin from Eli Lilly, pristinamycins IA and IIA from Rhône-Poulenc, spiramycin I from Specia, ivermectin was a gift from Merck Sharp & Dohme, and pimaricin from Gist-Brocades; polyethyleneglycol (PEG) 6000 and dextran T500 were provided by BDH and Pharmacia respectively.

Buffers used included: (A) 20 mM tris-HCl (pH 7.6), 10 mM $MgCl_2$, 100 mM NH_4Cl; (B) 20 mM tris-HCl (pH 7.6), 6 mM $MgCl_2$, 60 mM NH_4Cl; (C) 10 mM potassium phosphate (pH 7.6), 10 mM $MgCl_2$, 100 mM KCl.

Action of β-Mercaptoethanol on Rosaramicin

900 μl of 6 mM β-mercaptoethanol were added to 100 μl of aqueous 10^{-3} M rosaramicin or dihydro-

rosaramicin. The decrease in UV absorption was followed at 240 nm and ended after 1 hour of reaction at room temperature.

For preparative purpose, 11.6 mg, 2×10^{-5} mole of rosaramicin were dissolved into 400 μl of dimethylformamide, after which 75 ml of 6 mM aqueous β-mercaptoethanol were added. By the end of 4 hours at room temperature, and after checking for the completion of the reaction by thin-layer chromatography (aluminoxid 60F 254 neutral, Merck, with ethyl acetate - methanol, 18: 1 as an eluent), solvents were evaporated under reduced pressure or by lyophilization. A preparative TLC was done under the same conditions as the analytical one was. The yield of 11-(2-hydroxyethylthio)-10,11-dihydrorosaramicin was 8 mg (two spots on TLC).

IR ($CHCl_3$) 1725 cm^{-1}. NMR (90 MHz, C^2HCl_3) ppm: 1.36 and 1.43 (2 isomers), 2.20 (N$\langle$), HO–CH_2–CH_2–S, t at 2.88 and 3.91. No ethylenic protons at 6.3, 9.77 (–C–CHO).

Preparation of the Ribosomes

Escherichia coli MRE600 tight ribosomes were prepared by zonal centrifugation as described[1].

Determination of the Binding Parameters by Equilibrium Dialysis

Equilibrium dialysis was carried out as previously described[1] using the isotopic dilution method.

Determination of the Binding Parameters by Ethanol Precipitation

The conditions were previously described[2]: 150 μl volumes of standard buffer B containing a final concentration of 33% (v/v) ethanol were incubated with 70S ribosomes and the radioactive antibiotic for 45 minutes at 0°C. Ribosomes were precipitated under these conditions and pelleted by centrifugation for 10 minutes at $3000\times g$; 100 μl of the supernatant were withdrawn and counted in a Kontron scintillation counter, using Ready Solv TMMP (Beckman) as a scintillation fluid.

Determination of the Binding Parameters by Aqueous Two-phase Partitioning

The conditions were as described elsewhere[3] with some modifications: a dextran/PEG (7%/5%, w/w) biphasic system was used; 500 μl of PEG phase in buffer B were added to 500 μl of dextran phase in buffer B containing 70S ribosomes and the radioactive antibiotic. Samples were gently rocked at 20°C for 40 minutes and then centrifuged for 5 minutes at $3000\times g$. 100 μl of each phase were pipetted off, diluted three times with water to avoid precipitation problems, added to 5 ml of Ready Solv TMHP Beckman scintillation liquid and counted.

Synergism Experiments

Mueller-Hinton agar was used for the search for synergism by the agar diffusion method. An overnight culture of either *E. coli* MRE 600 or *Staphylococcus aureus* 209P was diluted to a final concentration of about 10^6 cells per ml for inoculating plates. Antibiotic discs were placed onto the surface of the agar with one of each antibiotic situated on either branch of an L. The plates were incubated overnight at 37°C. In the case of *E. coli*, the disc content was 1.5 μg for rosaramicin and 1.5 or 15 μg for puromycin and in the case of *S. aureus* 0.15 μg or 1.5 μg for rosaramicin and 15 μg for puromycin.

Results

Requirements for [^{3}H]Dihydrorosaramicin Binding

The first binding experiments were conducted in buffer A. To check whether these conditions were actually the best ones for 70S and 50S ribosomes, we studied the influence of various factors on the binding parameters. The method used was equilibrium dialysis.

a) Influence of Mg^{++}

The results are shown in Fig. 1. When Mg^{++} varies from 1 to 30 mM, there is a 25% variation in the binding of the antibiotic. The optimum value is around 6 mM for both 50S and 70S ribosomes. At this Mg^{++} concentration and above, the binding is always 10% better for 70S than for 50S. Below 6 mM this difference decreases, and at 1 mM Mg^{++} it disappears.

Fig. 1. Effect of $MgCl_2$ on [^{3}H]dihydrorosaramicin binding to *E. coli* 70S ribosome (□) and 50S subunit (○).

Equilibrium dialysis experiments were carried out in buffer A with varied $MgCl_2$ concentrations and using the following final concentrations: 65 nM [^{3}H]dihydrorosaramicin and 240 nM ribosome.

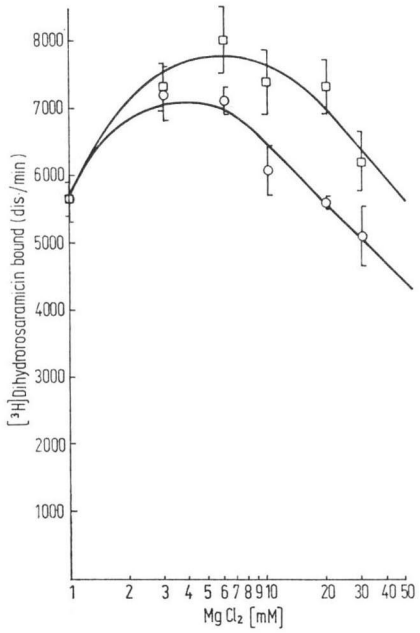
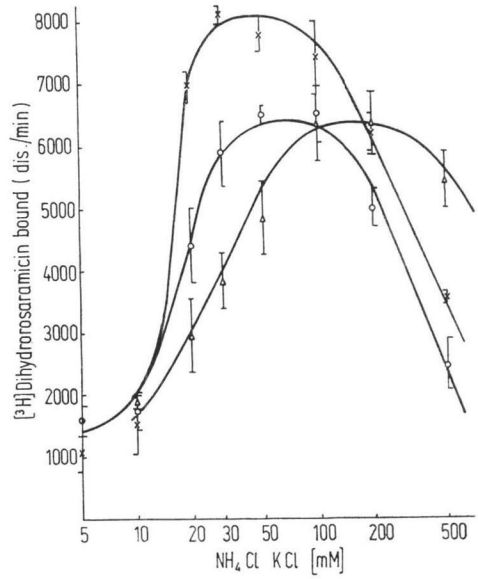


Fig. 2. Effect of monovalent cations on [^{3}H]dihydrorosaramicin binding to *E. coli* ribosomes.

The assay mixtures were the same as those described in Fig. 1 except that the buffer A was used with varied NH_4Cl or KCl concentrations: (×) NH_4Cl, 70S ribosome; (○) NH_4Cl, 50S subunit; (△) KCl, 70S ribosome.



b) Influence of Ionic Strength

Fig. 2 shows the great influence of NH_4^+ concentration on the binding of [^{3}H]dihydrorosaramicin to 70S and 50S ribosomes, and of K^+ on its binding to 70S ribosome. The optimum range for both 70S and 50S ribosomes is from 30 to 100 mM for NH_4^+ and from 100 to 300 mM for K^+. The maximum binding value is 20% higher for NH_4^+ than for K^+.

c) Influence of pH and Buffer

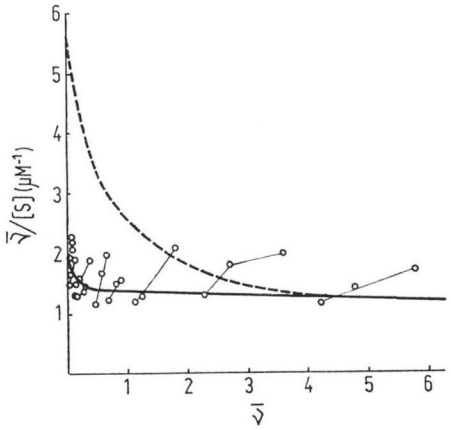
The experiments were conducted in tris - maleate buffer at pH levels between 6.4 and 8.3, the pH range where the ribosomes are stable[4]. The results (not shown) indicate that the amount of [^{3}H]dihydrorosaramicin bound to the 50S and 70S ribosomes remains essentially constant. Experiments conducted in phosphate buffer C at pH 7.6 give the same results as previously those described in tris - HCl buffer (pH 7.6)[1].

d) Influence of β-Mercaptoethanol

Fig. 3 shows that the SCATCHARD plots obtained from isotopic dilution method results without[1] and with β-mercaptoethanol (6 mM) are very different: we no longer observe the first part of the curve giving $K_D = 0.2 \times 10^{-6}$ M, n=1, but we do the second part. This corresponds to low affinity binding sites and gives the average value $K_D = 0.2 \times 10^{-4}$ M, n=25. This result prompted us to examine the possible reaction of β-mercaptoethanol on dihydrorosaramicin and rosaramicin. The kinetics of this reaction (not shown) was followed by ultraviolet measurement at 240 nm; this wavelength corresponds to the

Fig. 3. Effect of β-mercaptoethanol (6 mM) on [^{3}H]-dihydrorosaramicin binding to *E. coli* 70S ribosome.

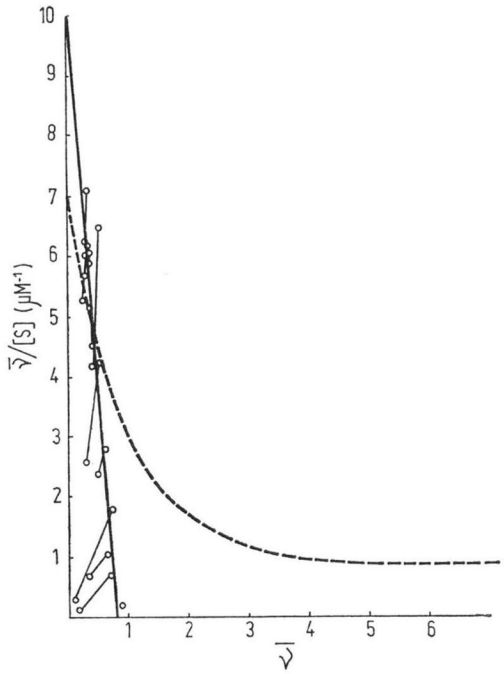
SCATCHARD plot was obtained from equilibrium dialysis results carried out in buffer A with 6 mM β-mercaptoethanol using the following concentrations: 240 nM 70S ribosome, 65 nM [^{3}H]dihydrorosaramicin and increasing concentrations of dihydrorosaramicin. (------) reference SCATCHARD plot obtained using buffer A.[1]



absorption of the chromophore $-C-C-C=\overset{|}{C}-C=O$ (with O) which completely disappears after 1 hour of reaction. Dithiothreitol (2 mM) has the same effect.

Fig. 4. SCATCHARD plot of two-phase partitioning data.

The experiments were carried out in buffer B using the following final concentrations: 192 nM 70S ribosome, 50 nM [^{3}H]dihydrorosaramicin and increasing concentrations of dihydrorosaramicin. (------) reference SCATCHARD plot obtained using equilibrium dialysis in buffer B.



The UV, IR and NMR spectra of the modified product indicate that it results from a MICHAEL type addition of the thiol to the α,β-unsaturated ketone. In our case, the TLC and NMR spectra (two signals for the methyl adjacent to the epoxide) suggest the presence of two isomers.

e) Influence of Temperature

The preceding results led us to use buffer B in further experiments. In this optimal buffer, the binding parameters found at 20°C were $K_D = 0.15 \times 10^{-6}$ M and n=0.9 for 70S ribosome (results not shown). The binding was also carried out at 4°C where the parameters were $K_D = 0.56 \times 10^{-6}$ M, n=1.1, even after 15 minutes of preincubation of the ribosomes at 37°C (results not shown). Because of problems of reproducibility at this temperature, equilibrium dialysis was performed at 20°C.

Ethanol Precipitation and Two-phase Partitioning

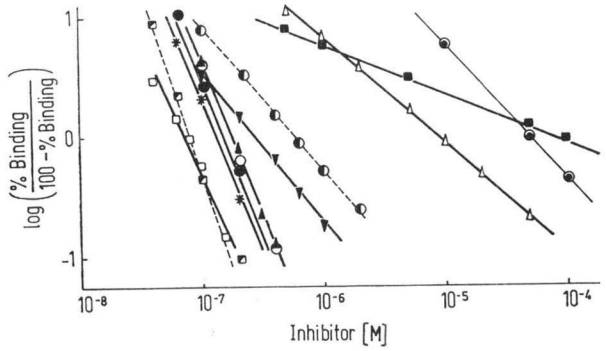
Table 1 shows that the binding parameters did not depend on the technique used: equilibrium dialysis, ethanol precipitation and two-phase partitioning. As expected from a previous paper[3], two-phase partitioning always gave the best results. It is also noteworthy that it only

Table 1. Influence of the technique used on the parameters of [^{3}H]dihydrorosaramicin binding to *E. coli* 70S ribosome.

Techniques used in buffer B	K_D	n
Equilibrium dialysis	20°C: 1.5×10^{-7} M	0.9
	4°C: 5.6×10^{-7} M	1.1
Ethanol precipitation	4°C: 5.2×10^{-7} M	1
Two-phase partitioning	20°C: 0.94×10^{-7} M	0.83

Fig. 5. Inhibition of [^{3}H]dihydrorosaramicin binding by different antibiotics.

The log plot for dihydrorosaramicin competition was obtained from the direct competition experiments carried out by equilibrium dialysis in buffer B and using the following concentrations: 240 nM 70S ribosomes, 65 nM [^{3}H]dihydrorosaramicin and increasing concentrations of inhibitors, spiramycin I (□), midecamycin (*), erythromycin (●), pristinamycin IIA (▲), pristinamycin IA (▼), pristinamycins IIA and IA in 10/1 ratio respectively (○), chloramphenicol (△), tobramycin (■), 11-(2-hydroxyethylthio)-10,11-dihydrorosaramicin (◒). Dotted lines report our previous competition results[1] obtained with rosaramicin (◩) and dihydrorosaramicin (◐).



leads to the first part of our usual SCATCHARD curve (Fig. 5). In our calculations, we naturally took into account the partition coefficient of [^{3}H]dihydrorosaramicin between the two phases:

$$K_P = \frac{\text{concentration in PEG}}{\text{concentration in dextran}} = 1.4.$$

Ability of Other Antibiotics to Inhibit the Binding of [^{3}H]dihydrorosaramicin to 70S Ribosomes

In order to obtain more information about the binding site of rosaramicin, competition experiments were performed using an equilibrium dialysis and isotopic dilution method with [^{3}H]dihydrorosaramicin as a marker. According to previous data[2], bound [^{3}H]dihydrorosaramicin concentration would not exceed 0.1 K_D. The competitors may be classified into two groups:

Molecules of the macrolide family which are C_{16}-membered: 11-(2-hydroxyethylthio)-10,11-

Fig. 6. Effect of puromycin and pimaricin on [^{3}H]dihydrorosaramicin binding to *E. coli* 70S ribosome.

Equilibrium dialysis experiments were carried out in buffer B using the following concentrations: 240 nM ribosomes, 65 nM [^{3}H]dihydrorosaramicin and increasing concentrations of antibiotic: puromycin (●), pimaricin (○), dihydrorosaramicin (---).

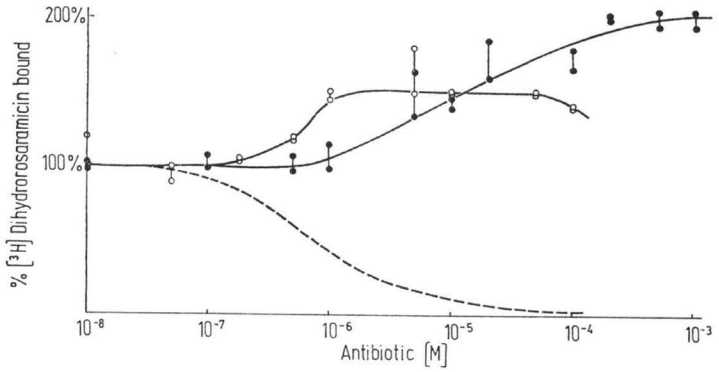


Table 2. Dissociation constants (K_D) and 50% inhibition concentrations (I_{50}) towards [^{3}H]dihydrorosaramicin and [^{14}C]erythromycin of several inhibitors of protein synthesis.

The values are either from the literature or determined by us using isotopic dilution method. I_{50} is the concentration of inhibitor necessary to remove 50% of the labeled antibiotic from its selective binding site determined from Fig. 5.

Antibiotic	$K_D(\times 10^{-7}$ M)	$I_{50}(\times 10^{-7}$ M)	
		[^{3}H]Dihydrorosaramicin	[^{14}C]Erythromycin
Rosaramicin	0.8	0.76	—
20,20-Dihydrorosaramicin	2	5.96	—
Thiorosaramicin	≃200	500	—
Leucomycin A_6	0.46 (13)	1.33	19 (12) 3.5 (13)
Spiramycin I	0.13 (13)	0.71	11 (13)
Erythromycin	0.1 (25) 0.2 (13)	1.50	19 (13)
Pristinamycin IA	0.5 (17) 7.1 (15) 4 (16)	2.78	200 (15)
Pristinamycin IIA	0.3 (17) 31 (28)	1.78	—
Pristinamycins: natural 10/1 mixture IIA/IA	 0.06 (17) 0.66 (16)	1.78	—
Chloramphenicol	1.04 (3) 1.02 (24) 4.8 (26)	89.1	10,000
Tobramycin	0.4 (3) 2 (27)	750	—
Puromycin	4800	no inhibition	—
Tetracycline	0.2 and 4.3 (3)	no inhibition	—

dihydrorosaramicin, midecamycin, spiramycin I, or C_{14}-membered (erythromycin), or various related products (ivermectin, pimaricin)

Other molecules which are well known for their binding on the bacterial ribosome (chloramphenicol, tobramycin, pristinamycins IA and IIA, tetracycline, puromycin).

Fig. 5 is the log plot of the inhibition experiments obtained from the direct competition curves (not shown); the K_D of different antibiotics, and their I_{50} towards [^{3}H]dihydrorosaramicin and [^{14}C]erythromycin binding (from the literature) can be seen in Table 2.

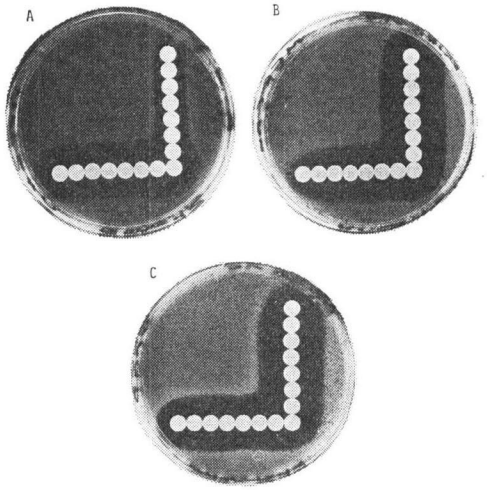
The effect of puromycin and pimaricin on dihydrorosaramicin binding is given in Fig. 6 which shows an enhancement of 100% and 50% respectively. Tetracycline and ivermectin, at concentrations ranging from 10^{-8} M up to 10^{-4} M and 10^{-9} M up to 6×10^{-4} M respectively do not influence the binding.

In Vivo Synergism Experiments Between Rosaramicin and Puromycin

As shown in Fig. 7, no synergism was observed either for *E. coli* MRE 600 or for *S. aureus* between rosaramicin and puromycin.

Fig. 7. Synergism experiments between rosaramicin (horizontal) and puromycin (vertical).

(A) *E. coli* MRE600, rosaramicin 1.5 μg, puromycin 1.5 μg; (B) *E. coli* MRE600, rosaramicin 1.5 μg, puromycin 15 μg; (C) *S. aureus* 209P, rosaramicin 0.15 μg, puromycin 15 μg.



Discussion

The influence of salts and pH on the binding of [^{3}H]dihydrorosaramicin to *Escherichia coli* ribosomes indicates that the optimal conditions are actually the same for both 50S and 70S particles. Nevertheless, these studies have shown that the amount of [^{3}H]dihydrorosaramicin bound was always 10% greater for 70S than 50S particles. No more difference is observed at 1 mM Mg^{++}, where 70S ribosome is dissociated into 30S and 50S subunits. This result allows us to suggest that, while it has no affinity for dihydrorosaramicin, the 30S particle somehow favors the binding by its presence in the 70S complex. The binding of dihydrorosaramicin to ribosomes is strongly dependent upon the K^+ or NH_4^+ concentration. Our results are in good agreement with those obtained in the case of erythromycin, the chief compound of the macrolide series[5,6,7]. It is well known[8] that it is better to store and use ribosomes in the presence of sulphydryl compounds. We have seen that they must be excluded from the buffers used, since they transform rosaramicin into the MICHAEL adduct: 11-(2-hydroxyethylthio)-10,11-dihydrorosaramicin. A similar reaction has been described in chalcomycin series[9]. The I_{50} from the competition assay using this product (Table 2) and the K_D from equilibrium dialysis of [^{3}H]dihydrorosaramicin performed in the presence of β-mercaptoethanol (Fig. 3) are both in the range of 0.5×10^{-4} M. This fact proves the MICHAEL adduct to be a poor competitor and to be formed during the β-mercaptoethanol supplemented dialysis.

The other techniques used in the binding studies confirm the results from equilibrium dialysis. Ethanol precipitation assays were carried out at +4°C and we should therefore compare it to equilibrium dialysis realized at the same temperature (Table 1). As shown in a previous paper[3], a two-phase partitioning system provides an environment where non-specific, weak affinity binding is not observed. This is particularly obvious here in the case of dihydrorosaramicin. Similarity of the Kas from both partitioning and equilibrium dialysis experiments indicates that the interpretation of the SCATCHARD curves according to ROSENTHAL[10] leads to exact values.

As two phase partitioning leads exclusively to the high affinity binding of dihydrorosaramicin, and β-mercaptoethanol supplemented dialysis shows only multiple low affinity binding, it is interesting to see that, taken together, both experiments lead to an artificial dissociation of our usual curve line (see on Figs. 3 and 4) into two parts. This result reinforces our prior hypothesis of one high affinity binding site[11].

The ability of compounds to affect dihydrorosaramicin binding to ribosomes could provide an estimate of their ability to interact with the rosaramicin binding site. In the macrolide series, we have shown that, regardless of their structure, all the antibiotics are potent inhibitors of dihydrorosaramicin binding and, as shown in Table 2, their I_{50} are closely similar to their K_D values. Thus, there can be little doubt that, even if the modes of action of these drugs are not necessarily the same, the ribosomal binding sites are closely related. These results are in good agreement with prior data concerning erythromycin[12,13,14]. In the particular case of pristinamycins, claimed to have different modes of action[15], they are both very good competitors of dihydrorosaramicin binding. Moreover, we observe that pristinamycin IIA com-

pound, having a lower K_D value than pristinamycin IA (0.3×10^{-7} M and 0.5×10^{-7} M respectively, this laboratory, results not published) is also a better competitor ($I_{50}=10^{-7}$ M and 3×10^{-7} M). It has been clearly demonstrated[16,17] that pristinamycins have a synergistic effect, since their K_D is shifted to 10^{-9} M for a pristinamycins IIA/IA ratio of 10/1. We have thus examined the possible synergistic effect of these molecules upon dihydrorosaramicin binding. Competition experiments carried out with the same concentration ratio fail to exhibit any synergism ($I_{50}=10^{-7}$ M), suggesting that the way by which pristinamycins compete for dihydrorosaramicin binding would not involve their own modes of action. All macrolide antibiotics, regardless of their structure are potent inhibitors of dihydrorosaramicin binding and, in retrospect, we are surprised that such an insignificant modification as the MICHAEL addition of β-mercaptoethanol transforms dihydrorosaramicin into so poor a competitor. One might be tempted to attribute an essential role in the binding properties to the α,β-unsaturated ketone system. But numerous basic 16-membered macrolides lacking this chromophore have antibacterial or binding properties comparable to those of rosaramicin[18]. This shows that the structure-biological activity correlation in this series is quite complicated. We have tested in the macrolide series, because of structural similarities, two non-antibiotic compounds. Ivermectin[19], despite its macrocyclic 16-membered lactone structure, has no effect on dihydrorosaramicin binding. Pimaricin[20], assayed because of its epoxide-ethylenic ketone system enhances the binding by 50%; this could not be necessarily attributed to a specific effect, since the structure of this C_{30} macrolide is only poorly related to the rosaramicin formula.

Potent inhibitors of dihydrorosaramicin binding are found exclusively among the macrolide antibiotics, which supposedly interfere with the peptidyltransferase site. However, chloramphenicol, which is also an inhibitor of the peptidyltransferase reaction, competes poorly against dihydrorosaramicin binding, indicating that this competition is not on the primary binding site of dihydrorosaramicin. Along the lines of these results, others[21] have reported that chloramphenicol is not able to displace erythromycin. On the other hand, the ability of erythromycin to displace chloramphenicol from its binding site has been largely discussed[22,23] so we will not deal here with the given interpretations. We have found (results not shown) that rosaramicin, even at concentrations as high as 10^{-4} M, is not able to displace bound chloramphenicol. This result reinforces the hypothesis that there is little or no interaction between the two sites[24].

When we assayed another peptidyltransferase inhibitor, puromycin, we were surprised to discover that rather than inhibit, it actually stimulates dihydrorosaramicin binding by about 100%. This lack of inhibition proves that dihydrorosaramicin and puromycin have different binding sites; this result correlates well with the above observations about chloramphenicol since puromycin and chloramphenicol are competitive inhibitors in respect with their binding on the ribosome[20]. We were unable to detect any *in vivo* synergism between rosaramicin and puromycin on Gram-positive and Gram-negative microorganisms. Nevertheless, the level of binding stimulation, observed *in vitro* on *E. coli* ribosome seems to be high enough to prompt us to develop further studies.

Tetracycline, known to bind to 50S subunit, does not influence the binding even at 10^{-4} M; the aminoglycoside tobramycin, whose effect upon ribosomes is quite different from the macrolides mode of action, is actually a very poor competitor. We can conclude that these two drugs have binding sites that are independent of the dihydrorosaramicin binding site.

This study should enhance our understanding of the binding of rosaramicin and the relationship between this molecule and other ribosome interacting antibiotics.

References

1) SIEGRIST, S.; J. LAGOUARDAT, N. MOREAU & F. LE GOFFIC: Mechanism of action of a 16-membered macrolide. Binding of rosaramicin to *E. coli* ribosome and its subunits. Eur. J. Biochem. 115: 323~327, 1981
2) FERNANDEZ-MUNOZ, R; R. E. MONRO & D. VAZQUEZ: Ribosomal peptidyl transferase, binding of inhibitors. *In* Methods in Enzymology. Vol. 20, pp. 481~490, Academic Press, New York, London, 1971
3) LE GOFFIC, F.; N. MOREAU, S. LANGRENÉ & A. PASQUIER: Binding of antibiotics to the bacterial ribosome studied by aqueous two-phase partitioning. Anal. Biochem. 107: 417~423, 1980
4) SABO, B. & A. S. SPIRIN: Dissociation of 70S ribosomes depending on the ionic strength, pH and temperature. Mol. Biol. Moscow 4: 618~827, 1970

5) TERAOKA, H.: A reversible change in the ability of *Escherichia coli* ribosomes to bind to erythromycin. J. Mol. Biol. 48: 511~515, 1970
6) TERAOKA, H.: Binding of erythromycin to *Escherichia coli* ribosomes. J. Antibiotics 24: 302~309, 1971
7) PESTKA, S.: Binding of ^{14}C erythromycin to *Escherichia coli* ribosomes. Antimicrob. Agents Chemother. 6: 474~478, 1974
8) ZAMIR, A.; R. MISKIN, Z. VOGEL & D. ELSON: The inactivation and reactivation of *Escherichia coli* ribosomes. *in* Methods in Enzymology, Vol. 30, pp. 406~426, Academic Press, New York, London, 1974
9) WESTLAND, R. D. & T. H. HASKELL: Synthesis of 11-substituted 10,11-dihydrochalcomycins and 2,3-dihydrochalcomycin, new antibacterial agents. Abstr. 13, 162nd Natl. Meet. Am. Chem. Soc., 1971
10) ROSENTHAL, H. E.: A graphic method for the determination and presentation of binding parameters in a complex system. Anal. Biochem. 20: 525~532, 1967
11) NORBY, J. G.; P. OTTOLENGHI & J. JENSEN: SCATCHARD plot: common misinterpretation of binding experiments. Anal. Biochem. 102: 318~320, 1980
12) ŌMURA, S.; A. NAKAGAWA, H. SAKAKIBARA, O. OKEKAWA, R. BRANDSH & S. PESTKA: Structure-activity relationships among the *O*-acyl derivatives of leucomycin, correlation of minimal inhibitory concentrations with binding to *Escherichia coli* ribosomes. J. Med. Chem. 20: 732~736, 1977
13) PESTKA, S.; A. NAKAGAWA & S. ŌMURA: Effect of leucomycins and analogues on binding [^{14}C]erythromycin to *Escherichia coli* ribosomes. Antimicrob. Agents Chemother. 6: 606~612, 1974
14) VAZQUEZ, D.: Binding to ribosomes and inhibitory effect on protein synthesis of the spiramycin antibiotics. Life Science 6: 845~853, 1967
15) DE BÉTHUNE, M. P. & K. H. NIERHAUS: Characterization of the binding of virginiamycin S to *Escherichia coli* ribosomes. Eur. J. Biochem. 86: 187~191, 1978
16) PARFAIT, R.; M. P. DE BÉTHUNE & C. COCITO: A spectrofluorimetric study of the interaction between virginiamycin S and the bacterial ribosome. Mol. Gen. Genet. 168: 45~51, 1978
17) ABBÉ, J.: Thèse de 3ème cycle. Université Paris VI, 1980
18) ŌMURA, S. & A. NAKAGAWA: Chemical and biological studies on 16-membered macrolide antibiotics. J. Antibiotics 28: 401~433, 1975
19) CHABALA, J. C.; H. MROZIC, R. L. TOLMAN, P. ESKOLA, A. LUSI, L. H. PETERSON, M. F. WOODS, M. H. FISHER, W. C. CAMPBELL, J. R. EGERTON & D. A. OSTLING: Ivermectin, a new broad spectrum antiparasitic agent. J. Med. Chem. 23: 1134~1136, 1980
20) GOLDING, B. T.; R. W. RICKARDS, W. E. MEYER, J. B. PATRICK & M. BARBER: The structure of the macrolide antibiotic pimaricin. Tetrahedron Lett. 1966: 3551~3554, 1966
21) FERNANDEZ-MUNOZ, R.; R. E. MONRO, R. TORRES-PINEDO & D. VAZQUEZ: Substrate and antibiotic binding sites at the peptidyltransferase center of *Escherichia coli* ribosomes. Chloramphenicol, lincomycin and erythromycin sites. Eur. J. Biochem. 23: 185~193, 1971
22) PESTKA, S.: Antibiotics as probes of ribosome structure: binding of chloramphenicol and erythromycin to polyribosomes; effect of other antibiotics. Antimicrob. Agents Chemother. 5: 255~267, 1974
23) PESTKA, S. & R. A. LE MAHIEU: Inhibition of [^{14}C]chloramphenicol binding to *Escherichia coli* ribosomes by erythromycin derivatives. Antimicrob. Agents Chemother. 6: 39~45, 1974
24) LANGLOIS, R.; C. R. CANTOR, P. VINCE & S. PESTKA: Interaction between the erythromycin and chloramphenicol binding sites on the *Escherichia coli* ribosomes. Biochemistry 16: 2349~2356, 1977
25) VINCE, R.; D. WEISS & S. PESTKA: Binding of *N*-substituted erythromycylamines to ribosomes. Antimicrob. Agents Chemother. 9: 131~136, 1976
26) GOLDBERG, R.; D. RINGER & S. CHLADEK: Inhibition of chloramphenicol binding to *Escherichia coli* 70S ribosomes by 2′(3′)-*O*-aminoacyl-dinucleotide phosphates derived from the aminoacyl-*t*-RNA acceptor terminus. Eur. J. Biochem. 81: 373~378, 1977
27) LE GOFFIC, F.; M. L. CAPMAU, F. TANGY & M. BAILLARGÉ: Mechanism of action of aminoglycoside antibiotics. Binding studies of tobramycin and its 6′-*N*-acetyl derivative to the bacterial ribosome and its subunits. Eur. J. Biochem. 102: 73~81, 1979
28) PARFAIT, R. & C. COCITO: Lasting damage to bacterial ribosomes by reversibly bound virginiamycin M. Proc. Natl. Acad. Sci. USA 77: 5492~5496, 1980
29) LESSARD, J. L. & S. PESTKA: Studies on the formation of transfer ribonucleic acid-ribosome complexes. J. Biol. Chem. 10: 6909~6912, 1972