# Interaction of Vernamycin A with *Escherichia coli* Ribosomes*

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ABSTRACT: The binding to *Escherichia coli* ribosomes of vernamycin A, an antibiotic which inhibits protein synthesis, was studied.

The binding of the antibiotic requires $K^+$ or $NH_4^+$, and $Mg^{2+}$, and is temperature dependent. Vernamycin A binds specifically to the 50S ribosomal subunits, and at saturation 1 mole of antibiotic is bound per mole of 70S ribosomes. The vernamycin A–ribosome complex formed in the presence of $K^+$ is very stable, but rapidly dissociates in the absence of the cation. Several antibiotics which are known to interact with the 50S ribosome subunit can prevent the binding of vernamycin A and can displace previously bound vernamycin A. An explanation of the mechanism of synergism between vernamycin A and Bα is proposed.

Studies on the binding of antibiotics to ribosomes have yielded information concerning the site and mode of action of drugs. These studies are not only useful because they tell us where the antibiotic works, but because they may be of help in mapping ribosomal binding sites involved in protein synthesis. For example, it may be possible, using this approach, to isolate specific proteins (or nucleic acids) from the ribosomes which bind specific antibiotics. Furthermore, this approach can also be used for genetic analyses of ribosomal proteins. One example of the latter approach is the finding that ribosomes isolated from erythromycin resistant strains of *Escherichia coli* which bind erythromycin poorly have an altered ribosomal protein (Otaka *et al.*, 1970).

A number of antibiotics which inhibit protein synthesis have been shown to interact directly with the 50S ribosomal subunit (Mao and Putterman, 1969; Vazquez, 1966, 1967). Among these antibiotics are those of the streptogramin[1] group, which include several related compounds. We have been interested in the mode and site of action of two antibiotics of the streptogramin group, synergistin (PA114) and vernamycin (Actor *et al.*, 1963; Celmer and Sobin, 1956; Laskin and Chan, 1965). The antibiotics are composed of two components, each of which is active alone in inhibiting growth and protein synthesis, but are synergistic in combination (Actor *et al.*, 1963; Celmer and Sobin, 1965; Ennis, 1965a,b). The structure of the A component has been described and it contains an oxazole nucleus (Delpierre *et al.*, 1966). The B component is a cyclic hexapeptide (Eastwood *et al.*, 1960; Vanderhaeghe and Parmentier, 1960).

Vernamycin A is a very potent inhibitor of bacterial growth and protein synthesis but is relatively ineffective against animal cells (Ennis, 1965b). It is a powerful inhibitor of *in vitro* protein synthesis, and has been shown to inhibit the puromycin reaction (Ennis, 1970; Pestka, 1970) and the binding of the Phe-tRNA oligonucleotide C-A-C-C-A(Phe) to ribosomes (Pestka, 1969). Vernamycin A is the best known inhibitor of these reactions. Its action has been located on the 50S subunit (Ennis, 1966). One other aspect of the action of vernamycin A is that it is synergistic in activity when mixed with vernamycin Bα (Actor *et al.*, 1963; Ennis, 1965a) (an antibiotic produced by *Streptomyces loidensis* at the same time as the A component). It is also interesting that [$^3$H]vernamycin A does not bind to purified calf brain ribosomes (unpublished data).

However, no direct studies have been done on the binding because radioactive vernamycin A was unavailable. The present investigation is a detailed study of the characteristics of the binding of [$^3$H]vernamycin A to ribosomes.

## Materials and Methods

*Preparation and Purification of* [$^3$H]*Vernamycin A.* [$^3$H]-Vernamycin A (2500 cpm/pmole) was prepared by New England Nuclear Corp. by catalytic tritium exchange. The [$^3$H]vernamycin was purified by chromatography on thin-layer plates made of silica gel HF using chloroform–methanol (95%–5%, v/v) to develop the chromatograms (Gosselinckx and Parmentier, 1962). The purity of the sample was determined in four ways: (1) the ultraviolet spectrum was identical with authentic vernamycin A's; (2) the purified [$^3$H]vernamycin A was as effective in inhibiting *in vitro* synthesis of polyphenylalanine as a known sample; (3) [$^3$H]vernamycin A inhibited the growth of susceptible bacteria at the same concentration as authentic vernamycin A; and (4) the [$^3$H]vernamycin A migrated as a single radioactive spot identical with that of the authentic antibiotic on thin-layer plates.

*Ribosomes.* Ribosomes were prepared from *Escherichia coli* B or MRE 600 and washed three times in a buffer containing 50 mM KCl–10 mM Tris-HCl (pH 7.8), and 10 mM magnesium acetate, or two times in the same buffer except that 1 M $NH_4Cl$ was added in place of KCl. Purified 50S and 30S subunits were kindly supplied by Dr. S. Pestka. Ribosomes (70S) were used exclusively except where indicated. Ribosomes washed in 1 M $NH_4Cl$ bound approximately 75% the amount of antibiotic as ribosomes washed in 50 mM KCl.

*Standard Assay Procedure.* The reaction mixture contained 100 mM KCl–10 mM magnesium acetate–10 mM Tris-HCl (pH 7.8), 15 pmoles of ribosomes, and 100 pmoles of [$^3$H]-vernamycin A ($2 \times 10^{-7}$ M final concentration, 2500 cpm/pmole) in a total volume of 0.5 ml. The reaction was initiated by the addition of ribosomes and was performed at 37°. It was terminated by cooling rapidly and diluting with 10 ml of cold buffer (as above). The contents of each tube were filtered through a Millipore filter (0.45 μ pore size) and washed with two 10-ml portions of the above buffer. The filters were placed in scintillation solution described by Bray (1960) and counted

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[1] The terminology of Vazquez (1966) will be used. Other antibiotics in this group are synergistin (PA114), vernamycin, mikamycin, ostreogrycin, and staphylomycin.

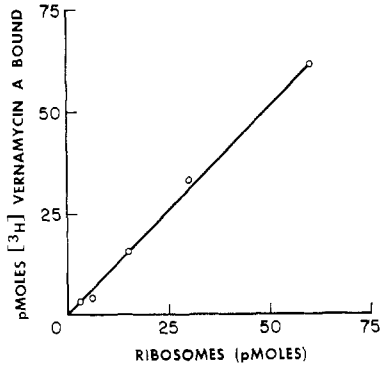


FIGURE 1: Dependence of binding of vernamycin A on ribosome concentration. The reaction was carried out under the standard conditions described in the Materials and Methods section, except that the indicated concentrations of ribosomes were used. Incubations were carried out at 37° for 15 min.

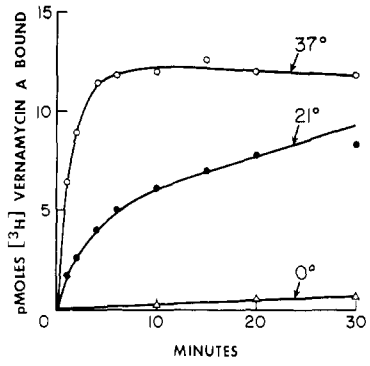


FIGURE 3: The effect of temperature on the rate of binding of vernamycin A. The reactions were carried out at 37 (○), 21 (●), and 0° (△), and the formation of the antibiotic–ribosome complex was determined at the indicated intervals as described in Materials and Methods.

using a Beckman LS-100 scintillation spectrometer. Since about 2% of input [$^3$H]vernamycin A binds to the Millipore filters even without ribosomes, controls without ribosomes were always run at the same time. This value was subtracted from each experimental reaction.

Similar binding was also observed using three other methods of analysis: (1) sedimentation through sucrose density gradients; (2) centrifugation and analysis of the pelleted ribosomes; and (3) gel filtration using Sephadex G-25. However, the Millipore filter technique was used in most experiments since it is rapid and easy to perform.

*Antibiotics and Chemicals.* The antibiotics used were gifts of numerous individuals and this is indicated in the acknowledgments. All other chemicals used were the best grade commercially available.

## Results

*Requirements for the Binding of Vernamycin A to Ribosomes.* The dependency of vernamycin A binding on ribosome concentration was studied. It was shown (Figure 1) that the binding of the antibiotic is proportional to the ribosome concentration. Furthermore, it appears that at all ribosome concentrations studied, the binding is approximately 1 mole/mole of ribosomes.

$K^+$ or $NH_4^+$ and $Mg^{2+}$ are needed for binding of vernamycin A to ribosomes (Figure 2). Optimum binding is observed at 100–200 mM $K^+$ or $NH_4^+$, although at lower concentrations $NH_4^+$ is more effective than $K^+$. The requirement for $Mg^{2+}$ shows a broad optimum ranging from about 1.5 to 10 mM; at higher concentrations $Mg^{2+}$ is slightly inhibitory. $Na^+$ does not support binding of the antibiotic nor does it inhibit in the presence of $K^+$.

Binding of vernamycin A to ribosomes is temperature dependent. As can be seen in Figure 3, binding at 37° is rapid and is complete within 5 min; at 21°, the rate of binding is one-half that at 37°, whereas at 0° essentially no binding is observed.

*Effect of Concentration of Vernamycin A on Binding to Ribosomes.* In this experiment the number of moles of antibiotic bound per mole of ribosomes was determined. It was shown (Figure 4) that, at saturating levels of the antibiotic, approximately 1 mole of antibiotic was bound per mole of ribosomes.

The dissociation constant of the ribosome–antibiotic complex was determined from the above data and is approximately $1.8 \times 10^{-8}$ M.

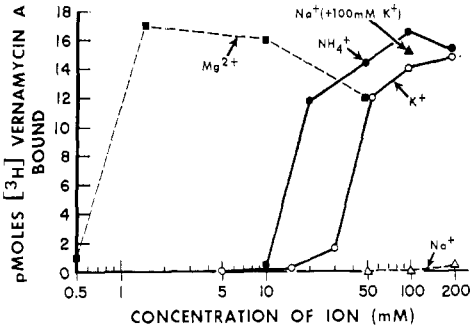


FIGURE 2: Ionic requirements for binding of vernamycin A to ribosomes. The reactions were carried out under the standard conditions as described in the legend to Figure 1, except that the concentrations of the indicated ions were varied when necessary. The ions not being studied were always kept constant at the concentrations indicated in Materials and Methods. The reactions were terminated as described in Materials and Methods, and the filters were washed using the same buffer as that in which the reaction was run. The results are an average of at least 3 separate determinations. Incubations were carried out for 15 min at 37°. (■), $Mg^{2+}$; (●), $NH_4^+$; (○), $K^+$; (▲), $Na^+$ (plus 100 mM $K^+$); (△), $Na^+$.

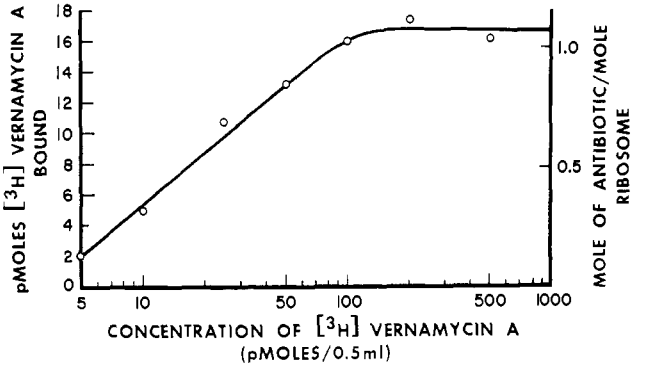


FIGURE 4: Effect of concentration of vernamycin A on binding to ribosomes. The reaction was carried out under the standard conditions as described in the legend to Figure 1, except that the [$^3$H]-vernamycin concentration was varied as indicated. The specific activity of the antibiotic was 2500 cpm/pmole in all the reactions. The results are an average of at least three determinations.

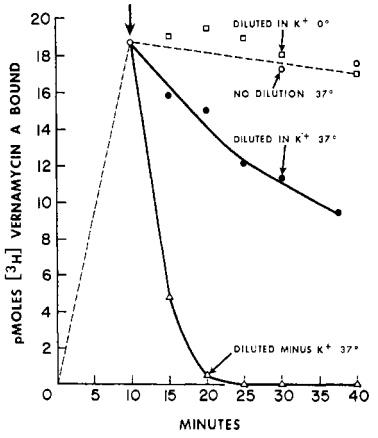


FIGURE 5: Stability of the ribosome–vernamycin A complex in the absence of $K^+$. The antibiotic–ribosome complex was made using the standard conditions described in Materials and Methods. At the times indicated by the arrow, aliquots of the reaction mixture were taken and treated as follows: (●) this portion was diluted 50-fold in the same buffer used in the reaction and incubation was continued at 37°; (□) this portion was diluted 50-fold as above, but incubated at 0°; (△) this aliquot was diluted 50-fold in the same buffer lacking $K^+$ and incubated at 37°; and (○) the last aliquot was not diluted and was incubated at 37°. The amount of [$^3$H]-vernamycin A bound to ribosomes at the indicated times was determined as described in the Materials and Methods section.

*Subunit Specificity of the Binding of Vernamycin A to Ribosomes.* Our previous studies indicated that the 50S subunit was inactivated by the antibiotic whereas the 30S subunit was unaffected (Ennis, 1966). Consequently, the subunit specificity of binding was determined. Both by Millipore filtration and by sucrose density gradient analysis, vernamycin A was found to bind only to the 70S ribosome or 50S subunits (data not shown). In one typical experiment, 1.13 pmoles of vernamycin A bound per pmole of 70S ribosomes, 1.16 pmoles/pmole of 50S ribosomes, and only 0.11 pmole/mole of 30S ribosome.

*Stability of the Ribosome–Vernamycin A Complex.* The stability of the ribosome–vernamycin A complex was studied under a variety of conditions. Our previous studies indicated that this interaction was reversible if $K^+$ was removed from the complex (Ennis, 1970). In the experiment outlined in Figure 5, [$^3$H]vernamycin was bound to ribosomes as previously described. Aliquots were then taken and treated as follows. One portion was diluted 50-fold in the same buffer used in the reaction and incubation was continued at 37°. One portion was diluted as above but incubated at 0°. Another aliquot was diluted 50-fold in the same buffer lacking $K^+$ and further incubated at 37°. The last aliquot was not diluted and was incubated at 37°. As can be seen, the vernamycin A–ribosome complex rapidly dissociates when diluted in buffer lacking $K^+$, whereas the complex dissociates slowly when diluted in buffer containing $K^+$ and incubated at 37°. No breakdown of the complex is observed when it is diluted and incubated at 0°.

The next experiment was designed to see whether [$^3$H]-vernamycin A could be displaced off the ribosome by dilution with 100-fold the concentration of nonradioactive vernamycin A or other antibiotics. As seen in Table I, [$^3$H]vernamycin can be displaced off the ribosome by vernamycin A, synergistin A, erythromycin, and spiramycin III. Erythromycin appeared to be more effective in this respect than the other antibiotics. All these antibiotics are also known to interact with the 50S ribosomal subunit (Vasquez, 1966). Other antibiotics which act on the 30S subunit, for example, chlorotetracycline and kanamycin, are unable to displace [$^3$H]vernamycin from the ribosome. Figure 6 shows the kinetics of the displacement reaction using nonradioactive vernamycin A to displace the [$^3$H]vernamycin A from the ribosome.

TABLE I: Ability of Antibiotics to Displace [$^3$H]Vernamycin A Bound to Ribosomes.[a]

| First Incubn | pmoles Bound | Second Incubn | pmoles Bound |
|---|---|---|---|
| 1. Control | 13.5 | (Diluted with) Water | 13.9 |
| 2. Control | 13.5 | Vernamycin A | 6.4 |
| 3. Control | 13.5 | Synergistin A | 9.5 |
| 4. Control | 13.5 | Erythromycin | 1.3 |
| 5. Control | 13.5 | Spiramycin III | 5.3 |
| 6. Control | 13.5 | Chlorotetracycline | 14.5 |
| 7. Control | 13.5 | Kanamycin | 13.9 |

[a] The first incubation was carried out under standard conditions for 10 min. Then aliquots of this reaction were added to tubes containing the indicated nonradioactive antibiotic ($2 \times 10^{-5}$ M final concentration) or water (control) to keep the volume constant. Incubations were carried out for an additional 30 min after which time the contents of each tube were filtered and washed, and the radioactivity bound to ribosomes was determined.

*Survey of Antibiotics Which Inhibit Binding.* A variety of antibiotics were studied for their ability to interfere with the binding of [$^3$H]vernamycin to ribosomes. In this series of experiments the standard reaction mixture contained, in addition to $2 \times 10^{-7}$ M [$^3$H]vernamycin A, an excess of unlabeled antibiotic to be tested. The antibiotic tested was considered ineffective in inhibiting binding when it produced less than 25% inhibition, at ten times the concentration of [$^3$H]vernamycin A.

It is important to note that the only antibiotics which interfered with vernamycin A binding to ribosomes (Table II) were antibiotics which interact with the 50S subunit and which have a similar mode of action as vernamycin A.

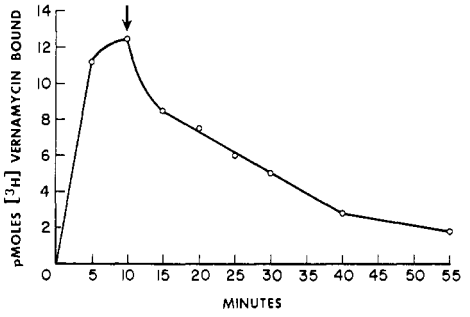


FIGURE 6: Ability of nonradioactive vernamycin A to displace $^3$H-vernamycin A bound to ribosomes. The antibiotic–ribosome complex was made using the standard conditions described in Materials and Methods. At the time indicated by the arrow, nonradioactive vernamycin A (final concentration $2 \times 10^{-5}$ M) was added. The ratio of nonradioactive to radioactive vernamycin A is 100-1. Samples were taken at the indicated intervals, and the amount of [$^3$H]-vernamycin A bound to ribosomes was determined as described in Materials and Methods.

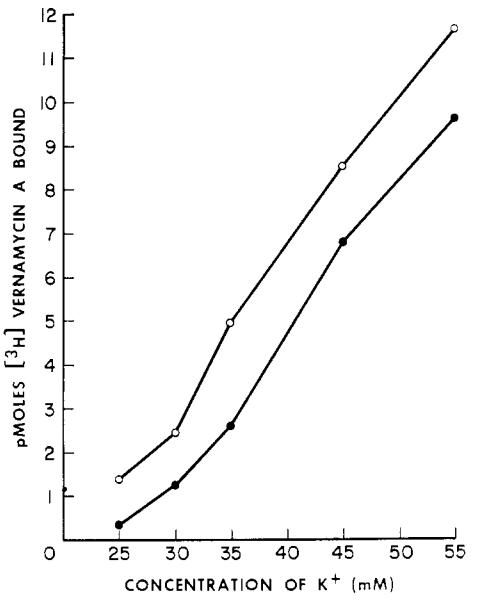


FIGURE 7: Stimulation of vernamycin A binding at low $K^+$ concentrations by vernamycin B$\alpha$. The reactions were carried out under standard conditions as described in the legend to Figure 1, either with (○) or without (●) nonradioactive vernamycin B$\alpha$ ($2 \times 10^{-6}$ M) and at the indicated $K^+$ concentrations. The amount of [$^3$H]vernamycin A bound to the ribosomes in each reaction was determined as outlined in Materials and Methods. The contents of each reaction were washed with the corresponding $K^+$-containing buffer. The results are an average of at least three separate determinations.

TABLE II: Antibiotics Which Inhibit Binding of [$^3$H]Vernamycin to Ribosomes.[a]

| Antibiotic | % of Control (100%) Bound | | | | |
|---|---|---|---|---|---|
| | $2 \times 10^{-7}$ M | $4 \times 10^{-7}$ M | $5 \times 10^{-7}$ M | $10^{-6}$ M | $2 \times 10^{-6}$ M |
| Carbomycin B (magnamycin) | 63 | 51 | | | 10 |
| Erythromycin | 55 | | 43 | 28 | 24 |
| Forocidin III | | | | | 58 |
| Leucomycin $A_3$ | 52 | 44 | | | 13 |
| Macrocin | 63 | | | | 13 |
| Neospiramycin III | 75 | 59 | | | 18 |
| Relomycin | | | | | 45 |
| Spiramycin III | 78 | | 43 | 35 | 23 |
| Synergistin A (PA114A) | 42 | | | 15 | 2 |
| Tylosin | 54 | 35 | | | 10 |
| Vernamycin A | 34 | | | | 14 |

[a] The standard reaction is outlined in the Materials and Methods section. The reaction was started by adding ribosomes and was incubated at 37° for 15 min.

Synergistin A, leucomycin $A_3$, tylosin, erythromycin, carbomycin, and macrocin all inhibit well. It may be seen again in Table II that except for synergistin A (which is probably the same as or certainly very similar to vernamycin A) all the other antibiotics which inhibit binding of vernamycin A are macrolides. Table III gives a list of the inactive antibiotics.

TABLE III: Antibiotics Which Do Not Inhibit Binding of [$^3$H]Vernamycin A to Ribosomes.[a]

Chloramphenicol Group
- Chloramphenicol
- D-(+)-*threo*-WIN-5094
- D-(+)-*threo*-WIN-5063
- D-*threo*-AMP-3
- D-(+)-*threo*-Thiocymetin

Macrolide Group
- Chalcomycin
- Lankamycin
- Methymycin
- Oleandomycin
- Picromycin

Lincomycin Group
- Celesticetin
- Clindamycin
- Lincomycin

Streptogramin B Group
- Synergistin (PA114) B
- Vernamycin B$\alpha$
- Viridogrisein (etamycin)

Other Antibiotics and Compounds
- Cephalosporin P1
- Deacylated *E. coli* B tRNA (780 pmoles)
- Poly(U) (200 $\mu$g)
- Aminoacyl-tRNA, *E. coli* B (200:1 ratio tRNA to [$^3$H]vernamycin)

Inhibitors of 30S Ribosomal Function
- Chlorotetracycline
- Hygromycin B
- Kanamycin A
- Paromomycin
- Spectinomycin
- Streptomycin
- Tetracycline

Other Inhibitors in 70S and/or 80S Ribosomal Systems
- Acetoxycycloheximide
- Amicetin
- Anisomycin
- Aurintricarboxylic acid
- Blasticidin S
- Bottromycin
- Cycloheximide
- Edeine
- Emetine
- Fusidic acid
- Gougerotin
- Pactamycin
- Puromycin
- Sparsomycin
- Thiostrepton

[a] Compounds which inhibit binding of [$^3$H]vernamycin A to ribosomes less than 25% at a concentration of $2 \times 10^{-6}$ M (10:1 ratio of compound to [$^3$H]vernamycin A) were considered noninhibitory. The standard reaction assay was used as described in Materials and Methods. The reaction was started by adding ribosomes and was incubated at 37° for 15 min.

TABLE IV: Effect of Preincubation Conditions on Ability of Ribosomes to Bind [$^3$H]Vernamycin.[a]

| Expt | Preincubn Condn (°C) | Incubn with [$^3$H]Vernamycin A (°C) | pmoles Bound |
|---|---|---|---|
| 1 | 37 | 0 | 5.6 |
| 2 | 0 | 0 | 1.1 |
| 3 | None | 37 | 14.4 |

[a] Ribosomes were preincubated for 15 min in the standard reaction mixture containing 100 mM $K^+$ but lacking the [$^3$H]vernamycin A, at the temperatures indicated above. The tubes were then cooled to 0° and the reaction was started by the addition of [$^3$H]vernamycin. The reactions were terminated as usual after 15 min and the amount of antibiotic bound to ribosomes was determined. The ribosomes in expt 3 were not preincubated and the reaction was run at 37° for 15 min.

TABLE V: Effect of Vernamycin Bα and Other Antibiotics on Vernamycin A Binding to Ribosomes: Displacement in the Absence of $K^+$.[a]

| First Incubn (Buffer Containing $K^+$) | pmoles Bound | Second Incubn | pmoles Bound |
|---|---|---|---|
| 1. [$^3$H]Vernamycin A | 13.7 | Diluted 50-fold with $K^+$-free buffer | 0.7 |
| 2. [$^3$H]Vernamycin A + unlabeled vernamycin Bα ($2 \times 10^{-7}$ M) | 13.0 | Diluted 50-fold with $K^+$-free buffer | 8.8 |
| 3. [$^3$H]Vernamycin A + unlabeled vernamycin Bα ($2 \times 10^{-6}$ M) | 11.5 | Diluted 50-fold with $K^+$-free buffer | 10.2 |
| 4. [$^3$H]Vernamycin A + unlabeled synergistin B ($2 \times 10^{-6}$ M) | 10.6 | Diluted 50-fold with $K^+$-free buffer | 9.2 |
| 5. [$^3$H]Vernamycin A + unlabeled viridogrisein ($2 \times 10^{-6}$ M) | 9.7 | Diluted 50-fold with $K^+$-free buffer | 0.9 |
| 6. [$^3$H]Vernamycin A + unlabeled viridogrisein ($2 \times 10^{-5}$ M) | 10.1 | Diluted 50-fold with $K^+$-free buffer | 6.1 |

[a] The first incubation was carried out under standard conditions in the presence of 100 mM $K^+$ and the indicated concentrations of nonradioactive antibiotic. Consequently, the final concentration of $K^+$ during the second incubation was 2 mM. Incubation was carried out for an additional 15 min at 37°. The contents of the tubes were filtered as usual except washed with buffer lacking $K^+$.

TABLE VI: Effect of Vernamycin Bα on [$^3$H]Vernamycin A Binding to Ribosomes: Displacement with Antibiotics.[a]

| First Incubation | pmoles Bound | Second Incubation | pmoles Bound |
|---|---|---|---|
| 1. [$^3$H]Vernamycin A + unlabeled vernamycin Bα | 12.5 | Control ($H_2O$ added) | 11.9 |
| 2. [$^3$H]Vernamycin A + unlabeled vernamycin Bα | 12.5 | Vernamycin A | 10.3 |
| 3. [$^3$H]Vernamycin A + unlabeled vernamycin Bα | 12.5 | Vernamycin Bα | 11.7 |
| 4. [$^3$H]Vernamycin A + unlabeled vernamycin Bα | 12.5 | Vernamycin A + Bα | 9.5 |
| 5. [$^3$H]Vernamycin A + unlabeled vernamycin Bα | 12.5 | Erythromycin | 11.8 |
| 6. [$^3$H]Vernamycin A + unlabeled vernamycin Bα | 12.5 | Spiramycin III | 11.3 |

[a] The first incubation was carried out under standard conditions for 10 min in the presence of $2 \times 10^{-6}$ M vernamycin Bα. Aliquots of this reaction were added to tubes containing the indicated nonradioactive antibiotic ($2 \times 10^{-5}$ M final concentration) or water (control) to keep the volume constant. Incubations were carried out for an additional 30 min after which time the contents of each tube were filtered and washed, and the radioactivity bound to ribosomes was determined.

*Effect of Preincubation of Ribosomes at 37° on Subsequent Binding of Vernamycin A at 0°.* Teraoka (1970) has shown that the affinity of ribosomes for erythromycin is dependent on the ribosomal conformation which can be altered by varying the $K^+$ concentration and the temperature. This seems also to be true of vernamycin A binding. The data presented in Table IV show that preincubation of ribosomes in 100 mM $K^+$ at 37° prior to incubation with [$^3$H]vernamycin at 0° changes the affinity of the ribosomes for the antibiotic at 0°. When binding at 0°, preincubation at 37° stimulates (expt 1) over the binding without preincubation (expt 2). Binding at 37° is more than that at 0° even after preincubation.

*Effect of Vernamycin Bα on Vernamycin A Binding to Ribosomes.* We had previously shown that vernamcyin Bα stimulated the interaction of vernamycin A with ribosomes (Ennis, 1966, 1970). The present studies were undertaken in an attempt to understand this phenomenon.

In this study, the binding reaction was run at various concentrations of $K^+$ with or without nonradioactive vernamycin Bα. Figure 7 shows that vernamycin Bα stimulated the binding of vernamycin A to ribosomes at low concentrations of $K^+$. No stimulation was observed when saturation of the ribosomes by 1 mole of vernamycin A/mole of ribosomes was reached.

The next experiments indicate that in the presence of vernamycin Bα, vernamycin A binding to ribosomes is irreversible on the basis of the test performed. In an earlier section experiments were described which showed that vernamycin A could be displaced off the ribosome either by diluting the antibiotic–ribosome complex in buffer lacking $K^+$ or by adding an excess of a variety of nonradioactive antibiotics. Similar experiments were performed, but this time the [$^3$H]-vernamycin A–ribosome complex was made in the presence of nonradioactive vernamycin Bα. The [$^3$H]vernamycin A bound to the ribosomes in this way was not displaced off the ribosomes by dilution with buffer lacking $K^+$ (Table V) or by addition of excess nonradioactive antibiotic (Table VI). See Figure 5 and Table I as a comparison for the same experiments done in the absence of vernamycin Bα. Synergistin B, which is similar if not identical with vernamycin Bα, is also active in this capacity, whereas viridogrisein (etamycin), another related antibiotic, is only effective at very high concentrations ($2 \times 10^{-5}$ M).

Occasionally significant inhibition (about 30%) of vernamycin A binding is observed when high concentrations of vernamycin Bα, synergistin B, or viridogrisein were used.

## Discussion

With the availability of radioactive vernamycin A, we have been able to investigate the properties of the binding of the antibiotic to ribosomes. The pertinent points we wish to make may be summarized as follows: (1). Vernamycin A binds preferentially to the 50S subunit. (2). $K^+$ or $NH_4^+$ and $Mg^{2+}$ are required. (3). The reaction does not occur at 0° (unless previously incubated at 37°), but takes place rapidly at 37°. (4). At saturation, 1 mole of antibiotic is

bound per mole of ribosomes. (5). The antibiotic–ribosome complex made in the presence of $K^+$ is stable. However, upon removal of the cation, the complex readily dissociates. (6). A variety of antibiotics (mostly macrolides) all of which interact with the 50S ribosomal subunit (Vazquez, 1966) can prevent the binding of vernamycin A and can also displace previously bound vernamycin A. None which interacts with the 30S subunit can prevent vernamycin A binding. Not all the antibiotics which affect 50S subunit function can affect vernamycin A binding. For example, although vernamycin A can inhibit chloramphenicol binding to ribosomes (Vazquez, 1966), the reverse was not observed. (7). Vernamycin Bα has a marked effect on the binding of vernamycin A to ribosomes. Probably the most interesting aspect of the present investigation is the last finding that vernamycin Bα also interacts with the ribosome. This interaction would appear to modify the ribosomes, by perhaps a conformational change, in some way enhancing the affinity of the ribosome for vernamycin A. Our study has shown that vernamycin Bα can stimulate vernamycin A binding when the conditions for binding are suboptimal (when $K^+$ is limiting) and also makes the vernamycin A which is bound in the presence of the B compound bind tenaciously to the ribosome. This result probably explains the mechanism of synergism. It would appear that vernamycin Bα acts by changing the conformation of the ribosome.

We have prepared [$^3$H]vernamycin Bα and are at present similarly studying the interaction of this compound with the ribosome and with other antibiotics.

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## CORRECTIONS

In the paper "On the Rate-Determining Step in the Action of Adenosine Deaminase," by Richard Wolfenden, Volume 8, No. 6, June 1969, page 2409, the following corrections should be made.

Page 2410, column 2, lines 29 and 30: the words "for attainment of the transition state" should be deleted.

Page 2412, column 1: after line 5 should be added the line: "transition state. The. . ."

In the paper "Reaction of Insulin with Ethyl Glycinate and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide," by Hitoshi Ozawa, Volume 9, No. 10, May 12, 1970, page 2158, the following corrections should be made.

Page 2161, column 2, line 11: ". . . using a 0.5–0.15 M sodium chloride gradient . . ." should read ". . . using a 0.05–0.15 M sodium chloride gradient . . ."

Page 2162, column 2, line 3: the heading "Studies on Separated A and B Chains of Peak 2" should read "Studies on Separated A and B Chains of Peak 2B." The same correction is also required on lines 5, 10, 19, and 22 of the same column.