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Interaction of Neomycin with Ribosomes and Ribosomal Ribonucleic Acid

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Neomycin binds ribosomes and ribosomal ribonucleic acid (rRNA) in vivo and in vitro producing changes detectable by increases in gel electrophoretic mobility. These changes were observed in gels that contain ethylenediaminetetraacetic acid or no added magnesium ion. The progressive increase in gel electrophoretic mobility with increasing antibiotic concentrations suggests that neomycin is binding at multiple sites on RNA. The binding was reversible but sufficiently stable to survive dialysis and electrophoresis. It is proposed that bound neomycin stabilizes the ribosome and RNA structures, restricting the unfolding of the particles during electrophoresis and thus allowing for a more rapid migration in the gel. Gentamicin produced an effect similar to that of neomycin. Paromomycin, differing from neomycin by only one amino group, had considerably less effect on ribosome and rRNA mobilities. The binding of neomycin to rRNA improved the linearity of the plot of log molecular weight versus mobility and thus may be of benefit in providing a more accurate estimation of molecular weights of large RNAs.

Neomycin, one of the aminoglycoside antibiotics, is an effective inhibitor of protein synthesis (14, 18). Its mode of action at the molecular level is less well understood than for many other antibiotics. Misreading of messenger ribonucleic acid (mRNA) in in vitro experiments has been detected at neomycin concentrations as low as 6.5×10^{-8} M (8). A progressive increase in misreading was noted over a wide concentration range, followed by a striking inhibition of translation at about 10^{-5} M. These observations suggest that neomycin is binding at multiple sites on the ribosome, each of which contributes to misreading (8). However, the complete inhibition of translation at high concentrations has not been explained.

Neomycin is positively charged and binds tightly to RNA (1). Although multiple neomycin binding sites in ribosomes could account for the pleiotropic effects of neomycin, until now there has been no direct evidence of such binding. Here we present evidence that neomycin binds to multiple sites on ribosomes, stabilizing the structure in a manner that is detectable by acrylamide gel electrophoresis. Furthermore, comparative studies with related aminoglycoside antibiotics provide some insight into both the mechanism by which neomycin may inhibit translation and the functionally important sites on the antibiotic molecule involved in the process.

MATERIALS AND METHODS

Pancreatic deoxyribonuclease (DNase) I (electrophoretically purified, ribonuclease [RNase]-free) was the product of Worthington Biochemical Corp. Egg-white lysozyme (three-times crystallized and lyophilized) was purchased from Mann Research Corp. Brij 58 (polyoxyethylene acetyl ether) was a gift of Atlas Chemical Industries, Inc. Acrylamide (catalog no. 5521), *N,N'*-methylenebisacrylamide, dimethylaminopropionitrile, and Stains-All (catalog no. 2718) were obtained from Eastman Organic Chemicals. Sea-Kem agarose was purchased from Marine Colloids Inc., Rockland, Maine. Reagent-grade chemicals were used throughout. Chloramphenicol, tetracycline, streptomycin, neomycin, erythromycin, polyuridylic acid ([poly(U)], polyadenylic acid [poly(A)], and tris(hydroxymethyl)aminomethane (Tris) (Sigma 7-9) were purchased from Sigma Chemical Co. Kanamycin, paromomycin, ribostamycin, and gentamicin were gifts from J. Davies, University of Wisconsin. MS 2 RNA was a gift from M. vonMontagu, State University of Ghent. Yeast ribosomal RNA (rRNA) was a gift from Terry Helser, Brown University. Rat liver rRNA and ribosomes were a gift from Roland Greene, Medical College of Philadelphia.

Bacteria and culture conditions. Cells of *Escherichia coli* B strain AS-19 (17) were grown at 37°C in L-broth medium with reciprocal shaking in a water bath. Under these conditions, cultures grew with a doubling time of 25 min.

Preparation of ribosomes and RNA. Cultures were harvested during the logarithmic phase of growth

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at a cell density of approximately 2×10^8 per ml by using rapid chilling on ice and lysed by a freeze-thaw lysozyme procedure (10). Cell debris was removed from the ribosome-containing lysate by a low-speed centrifugation. RNA was extracted from the lysate as described previously (6).

RNA fingerprint analysis. After polyacrylamide gel electrophoresis of RNA with and without added neomycin, the RNAs were isolated from the gel, precipitated, and digested with RNase T1 or pancreatic RNase as described elsewhere (2, 15). The resulting oligonucleotides were separated by two-dimensional paper electrophoresis (fingerprinting), as described by Sanger et al. (15).

Gel electrophoretic conditions. The preparation of the gels and the electrophoretic techniques were exactly as described previously (6, 9, 13) by using the vertical gel cell of E. C. Apparatus Corp., St. Petersburg, Fla. The gels were stained with Stains-All (5).

Analysis of RNA by sucrose density gradient centrifugation. Total cell RNA (5.5 mg) in 1.0 ml of 10 mM Tris-hydrochloride–150 mM NaCl buffer (pH 7.2) was combined with 2 mg of neomycin sulfate at 0°C for 15 min, precipitated with 3 volumes of ethanol, and redissolved in the same buffer before layering it on a 37-ml 5 to 20% (wt/vol) linear sucrose density gradient. A control sample, without neomycin, was similarly prepared, and the two samples were centrifuged at 27,000 rpm for 13 h in a Beckman L2-65B ultracentrifuge using an SW27 rotor at 3°C. The gradients were fractionated, and profiles were recorded by an ISCO fractionator with a UA-4 absorbance monitor. Fractions of 0.75 ml were collected.

RESULTS

In vivo effects of neomycin on gel electrophoretic mobility of ribosomes and rRNA. The binding of neomycin to ribosomes and RNA in vivo was examined by gel electrophoresis of samples from neomycin-treated cells. Neomycin was added to cells in log phase of growth; at intervals thereafter, the cells were harvested and washed free of neomycin, and the ribosomes and rRNA were isolated. Samples were electrophoresed into an agarose-acrylamide gel (Fig. 1). An increase in mobility was noted in both the ribosomal subunits and the rRNA's. The maximum increase was achieved within 30 min of addition of neomycin, coinciding with the cessation of all cell growth as measured by an absorbancy at 436 nm. No alteration in mobility of small RNAs (5S and transfer RNA [tRNA]) was detected in a 10% acrylamide gel (data not shown).

The neomycin-induced increase in electrophoretic mobility of rRNA could result from either a cleavage of the RNA or a significant conformational effect on secondary or tertiary structure, which would more than offset the molecular weight increase introduced by the bound antibiotic (neomycin molecular weight, 614). The former possibility was ruled out by RNA fingerprint analysis of 16S rRNA from ^{32}P-labeled control and neomycin-treated cells. No differences in the RNA fingerprint patterns were detected. The 3′- and 5′-containing oligonucleotides of mature 16S rRNA were present in both samples (data now shown).

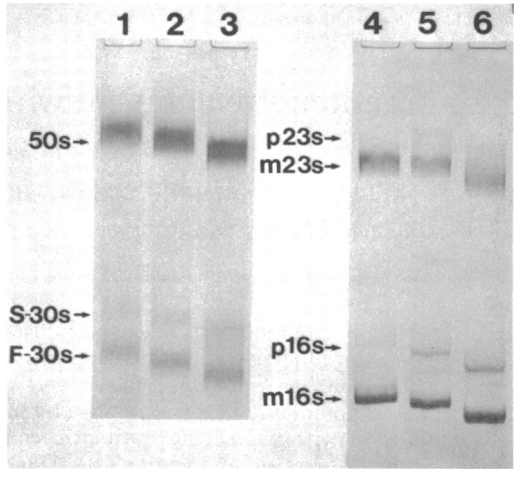


FIG. 1. *Comparison of electrophoretic mobilities of control and neomycin-treated ribosomal subunits and rRNA. An exponentially growing culture of E. coli AS19 at a cell concentration of approximately 2 × 10⁸ cells/ml received 300 μg of neomycin per ml. Samples of 40 ml were removed at 0, 10, and 30 min, washed once in lysozyme solution, and lysed. The ribosome-containing lysates (columns 1–3) and RNA extracts of the lysates (columns 4–6) were subjected to electrophoresis in a 3% acrylamide—0.5% agarose gel in Tris-EDTA-borate buffer (pH 8.3) for 6 h at 200 V and 0°C as described in the text. The gel was stained with Stains-All. Exposure to neomycin was for 0 (columns 1 and 4), 10 (columns 2 and 5), and 30 (columns 3 and 6) min. The bands are labeled p (precursor) and m (mature) for 23S and 16S rRNA (6). S-30S and F-30S represent the two forms of the 30S subunit (4, 17). Note accumulation of p16S rRNA during neomycin treatment.*

In vitro effect of neomycin. Neomycin was added to *E. coli* ribosomes and RNA in an attempt to duplicate the effects observed in vivo. An increase in gel electrophoretic mobility of rRNA was noted in vitro, with the extent of the increase being dependent upon the concentration of neomycin added (Fig. 2 and 3). At concentrations greater than 1 μg of neomycin per 5 μg of total cell RNA, there was no further increase in electrophoretic mobility (Fig. 3). At 20 μg of neomycin per 20 μl, slower migrating aggregates were noted (arrow, Fig. 2, column 4). A similar in vitro effect of neomycin was observed using ribosomes in a cell-free lysate (Fig. 3) and purified ribosomes isolated from a sucrose gradient (data not shown). The ribosome concentration used in Fig. 3 was twice that of the rRNA

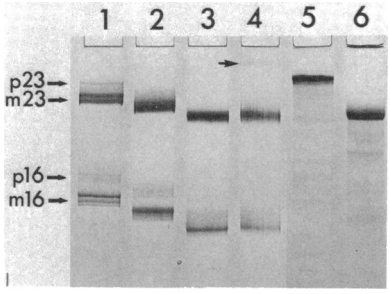


FIG. 2. *Effect of neomycin on the electrophoretic mobilities of rRNA and MS2 RNA. Neomycin was added to E. coli total cell RNA (5 μg) in 20 μl of 25 mM Tris-hydrochloride–50 mM NaCl–2 mM EDTA buffer (pH 7.0) and to MS2 RNA (3 μg) in 20 μl of 10 mM Tris-hydrochloride–10 mM $MgCl_2$ buffer (pH 7.2) for 15 min at 0°C before electrophoresis into a gel identical with that of Fig. 1. Columns 1–4: Cell RNA treated with 0 μg (column 1), 0.2 μg (column 2), 2.0 μg (column 3), and 20.0 μg (column 4) of neomycin. Columns 5 and 6: MS2 RNA treated with 0 μg (column 5) and 10.0 μg (column 6) of neomycin.*

concentration used (0.5 mg/ml, 10 μl per column), thus accounting for the apparent quantitative difference in response to neomycin.

Comparison of in vitro effects of neomycin with other antibiotics and polyamines. Several antibiotics were compared with neomycin for their abilities to affect the gel electrophoretic mobility of ribosomes and rRNA. Figure 3 shows the effects of paromomycin and ribostamycin, antibiotics very similar in structure to neomycin (Fig. 4). The replacement of a single amino group in neomycin with an hydroxyl group in paromomycin reduced considerably the ability of the antibiotic to alter the electrophoretic mobility of the ribosomes or rRNA. The absence of the 6 amino-L-glucosamine group in ribostamycin abolished all detectable effects of the antibiotic on RNA mobility.

Additional antibiotics were tested for their abilities to alter the electrophoretic mobilities of both ribosomes and rRNA as in Fig. 3. Of those tested, only gentamicin C_{1a} produced an effect equivalent to that observed with neomycin, and

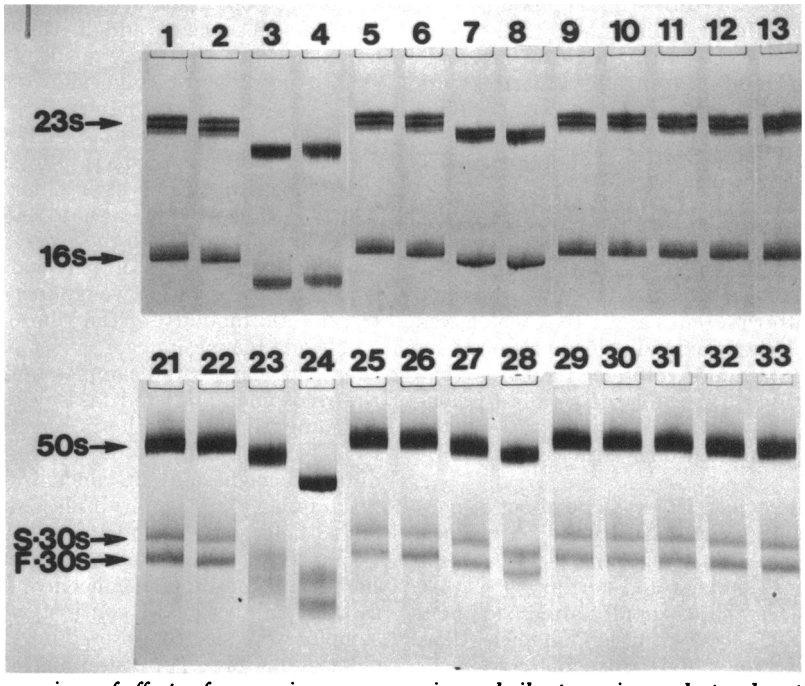
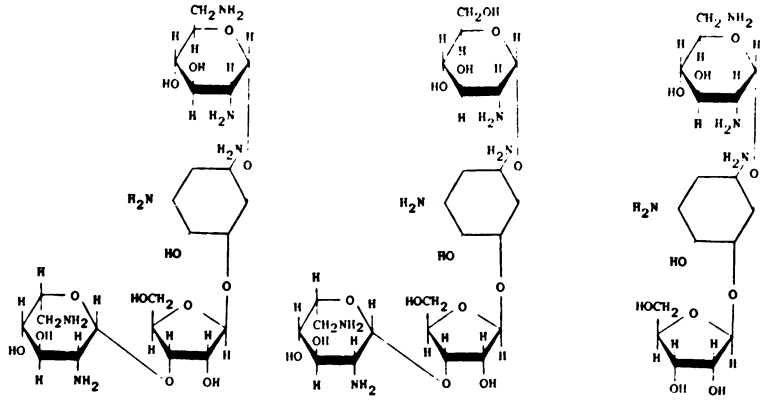


FIG. 3. *Comparison of effects of neomycin, paromomycin, and ribostamycin on electrophoretic mobility of RNA and subunits. Neomycin, paromomycin, and ribostamycin were added to E. coli total cell RNA (5 μg) as in Fig. 2 and to a ribosome-containing cell lysate of E. coli (approximately 10 μg of total cell RNA) for 15 min at 0°C before electrophoresis into a gel identical with that of Fig. 1. Columns 1, 5, 9, and 13: Cell RNA without antibiotics. Columns 2–4: Cell RNA treated with 0.1 μg (column 2), 1.0 μg (column 3), and 10 μg (column 4) of neomycin. Columns 6–8 and 10–12: Cell RNA treated with equivalent concentrations of paromomycin and ribostamycin, respectively. Columns 21, 25, 29, and 33: Ribosomes without antibiotics. Columns 22–24: ribosomes treated with 0.1 μg (column 22), 1.0 μg (column 23), and 10 μg (column 24) of neomycin. Columns 26–28 and 30–32: Ribosomes treated with equivalent concentrations of paromomycin and ribostamycin, respectively. The highest concentration (10 μg) of each antibiotic was in excess of that amount necessary to produce maximum increase in electrophoretic mobility of RNA and ribosomes.*



Neomycin B Paromomycin Ribostamycin

FIG. 4. *Structures of neomycin, paromomycin, and ribostamycin.*

then, only at an antibiotic concentration about 5 to 10 times greater than with neomycin. Gentamicin A, which lacks an amino group situated at one "end" of the three-ring compound, had essentially no effect on ribosome or rRNA mobility. Gentamicin B, lacking a more "internal" amino group, produced an effect intermediate between gentamicins A and C_{1a}. Other antibiotics tested, in order of decreasing maximal effectiveness, were paromomycin, kanamycin B, streptomycin, and kanamycin A. No effect was observed with kanamycin C, erythromycin, chloramphenicol, or tetracycline.

The polyamine spermine (at a 1 mM or greater concentration) produced a mobility change in both ribosomes and rRNA which was about 66% of the maximal effect produced by neomycin. Spermidine produced only a very slight effect and putrescine, the smallest polyamine tested, had no effect, although it is reported to alter the conformational structure of rRNA isomers (12).

mRNA and small RNAs. The effect of neomycin was not restricted to rRNA's. Viral RNA from the phage MS2, which reportedly contains considerable secondary and tertiary structure (11), migrated twice as rapidly after neomycin treatment (Fig. 2). Synthetic polynucleotides such as poly(U) and poly(A) also migrated faster with neomycin. However, neomycin (or paromomycin) added to small RNAs (6S, 5S, and tRNA) in vitro as opposed to in vivo actually retarded their electrophoretic mobilities (Fig. 5). A decrease in mobility of double-stranded deoxyribonucleic acid was also observed in the presence of added neomycin. Thus the observed increase in electrophoretic mobility in the presence of neomycin was limited to larger RNAs.

Role of magnesium ions in determining the effect of neomycin on ribosome mobility. The importance of magnesium ions in determining ribosome mobility in gels in the presence and absence of added neomycin was examined. Ribosomes were suspended in buffers containing different magnesium ion concentrations or ethylenediaminetetraacetic acid (EDTA). Neomycin was then added to fractions, and all samples were electrophoresed into gels containing either a Tris-hydrochloride buffer (no added EDTA or magnesium ion) or a Tris-EDTA-borate buffer. In the Tris-hydrochloride-buffered gel the effect of increasing the magnesium ion concentration was to increase the electrophoretic mobility of the ribosomes (Fig. 6). The same magnesium ion effect persisted with the neomycin-treated samples but, in all cases, the ribosomes migrated faster than the corresponding samples without neomycin. In the EDTA-containing gel, the ribosome samples in different magnesium ion concentrations showed no difference in mobility unless neomycin was added before electrophoresis. Again among the neomycin-treated samples, the ribosomes in the highest magnesium ion concentrations migrated farthest into the gel. In contrast, when the same samples were electrophoresed into a Tris-hydrochloride-buffered gel containing 5 mM magnesium chloride, no effect of neomycin was observed (data not shown).

Effect of neomycin on the association of 5S rRNA with the 50S subunit in EDTA-containing gels. Electrophoresis of ribosomes in an EDTA-containing gel causes dissociation of the subunits and release of 5S RNA (5). A two-dimensional gel electrophoresis method, which involves soaking the first-dimension gel in

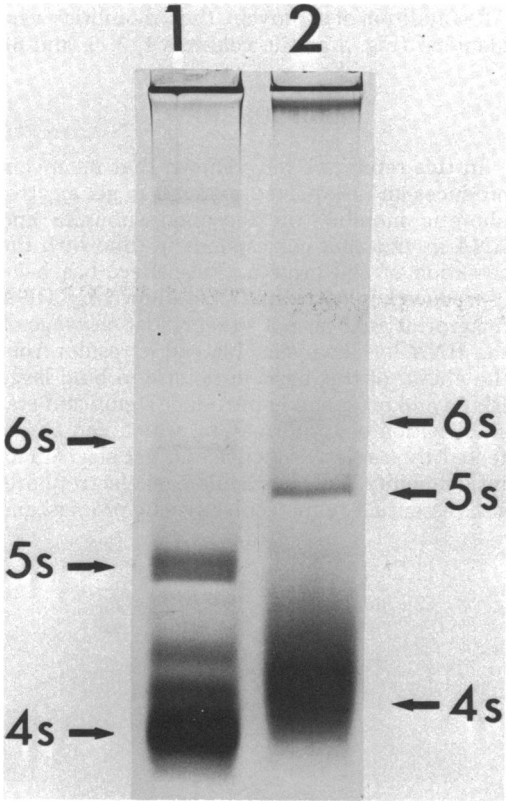


FIG. 5. *Effect of neomycin on the electrophoretic mobilities of small RNAs of E. coli. Control and neomycin-treated samples of Fig. 2 (columns 1 and 4, respectively) were electrophoresed into an 8% acrylamide gel in Tris-EDTA-borate buffer (pH 8.3) for 4 h at 200 V and 0°C. Samples: Control RNA (column 1), neomycin-treated RNA (column 2).*

sodium dodecyl sulfate and re-electrophoresis of the RNAs into a 10% gel to identify 5S RNAs (5) confirmed the lack of association of this small RNA with the two subunits. However, addition of neomycin to the ribosomes before electrophoresis caused the 5S RNA to remain associated with the 50S subunit as identified by the two-dimensional gel method (data not shown).

Neomycin and ribosomal proteins. The effect of the interaction of neomycin with ribosomal subunits persisted in the rRNA after removal of the ribosomal proteins by sodium dodecyl sulfate-phenol extraction. The extracted rRNA retained the increased mobility characteristic of the subunits (Fig 1). This suggests, although it does not prove, that neomycin interacted primarily with the rRNA rather than with the ribosomal proteins in the ribosome. It should also be noted that neomycin did not influence the association of ribosomal protein S1 with 30S subunits. The relative amounts of the two forms of 30S subunits (which differed by the presence or absence of S1) (4, 17) were unaffected by addition of neomycin (Fig 1, 3, and 6).

Reversibility of neomycin binding to RNA. The reversibility of neomycin binding to RNA was tested by mixing 16S rRNA's which had or had not been preincubated with neomycin. Treated and untreated 16S rRNA's were isolated by sucrose gradient density centrifugation. The sedimentation values of the 16S and 23S rRNA species of neomycin-treated RNA were noticeably faster than those of control RNAs (Fig. 7). Equal amounts of 16S rRNA from the two gradients were combined briefly at 0°C and then subjected to electrophoresis in EDTA-containing gels along with simultaneous electrophoresis of the two RNAs separately. The mobilities of the two separate 16S RNA (or 23S rRNA) samples differed as expected, but the mixture of the two samples resulted in a conver-

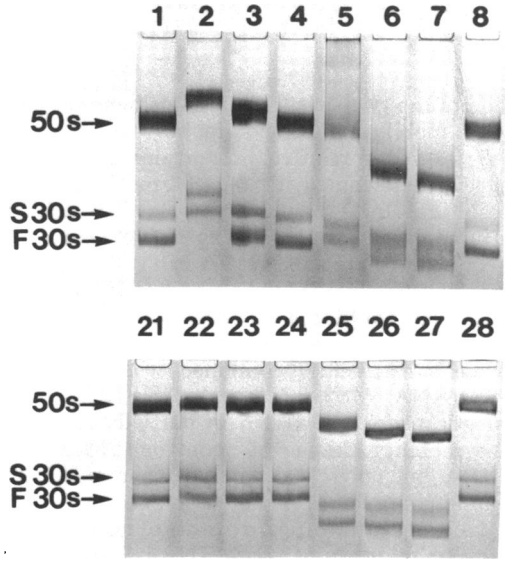


FIG. 6. *Role of magnesium ion in ribosome samples in determining the effect of neomycin on ribosome electrophoretic mobility. Ribosomes (10 μg) were suspended in 30 μl of 25 mM Tris-acetate buffer (pH 7.4) with the following additional components: 8 mM EDTA (column 2), no added components (column 3), 8 mM $MgCl_2$ and 50 mM KCl (columns 1, 4, and 8). Samples identical to columns 2, 3, and 4 received neomycin (7 μg) for 10 min at 0°C before electrophoresis in columns 5, 6, and 7, respectively. The samples were electrophoresed into 3% acrylamide-0.5% agarose gel for 6 h at 200 V and 0°C in Tris-acetate buffer (pH 7.4) (top gel) and in Tris-EDTA-borate buffer (pH 8.3) (bottom gel). Samples in columns 21-28 correspond to those in column 8. Gels were stained with Stains-All.*

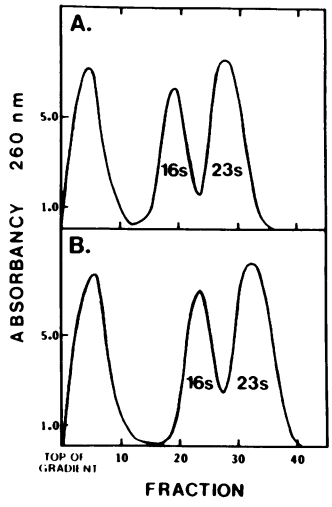


FIG. 7. *Effect of neomycin on sedimentation of rRNA. E. coli total cell RNAs, pretreated with and without neomycin, were separated by sucrose density gradient centrifugation as described in the text. (A) Control RNA; (B) neomycin-treated RNA.*

sion of the two species to a single band of RNA with mobility intermediate between the two (Fig. 8). The relative proportions of the two species determined the relative mobility of the resulting single band. This was also demonstrated for gentamicin and spermine. Dialysis of neomycin-treated 16S rRNA in Tris-hydrochloride buffer (pH 7.6) for 12 h at 3°C did not alter its gel electrophoretic mobility, nor did it restrict its ability to convert to a slower migrating form when subsequently combined with untreated 16S rRNA. MS2 RNA and 23S rRNA could substitute for 16S rRNA in partially reversing the mobility change of neomycin-treated 16S rNRA, but poly(U) and poly(A) had only minimal effects even when added in excess.

Neomycin as an aid in determining molecular weights of large RNAs. The molecular weight of RNA can be estimated by a plot of log molecular weight versus gel electrophoretic mobility (3, 13). Mobility values for large RNAs from several organisms were determined in EDTA-containing gels with and without added neomycin and plotted as a function of molecular weight. The data in Fig. 9 show that a more linear relationship was achieved for the set of RNAs treated with neomycin than for the untreated RNA. A striking example of the effect of neomycin was seen with MS2 RNA, the viral mRNA which has a molecular weight very similar to that of 23S rRNA but a gel electrophoretic mobility of about one-half that of 23S rRNA. After addition of neomycin, their mobilities were identical (Fig. 2 and 9, columns 1, 3, 5, and 6).

DISCUSSION

In this report we have shown that neomycin produces an unexpected increase in gel electrophoretic mobility of ribosomal subunits and RNA in vivo that corresponds in time with the cessation of cell growth. This effect has been reproduced in vitro and was shown by RNA fingerprint analysis not to represent cleavage of the RNA by neomycin. Instead it results from the ability of this basic antibiotic to bind large RNAs and presumably introduce significant stastabilization of conformation, which can be detected by a relative increase in gel electrophoretic mobility. Polyacrylamide gel electrophoresis is essentially a molecular sieving process, and

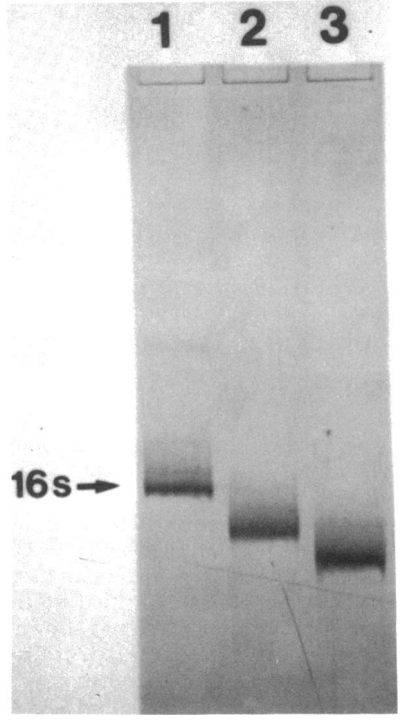


FIG. 8. *Effect of combining neomycin-treated and untreated 16S rRNA isolated from sucrose gradients. Portions of 16S rRNA from the two gradients of Fig. 7 were electrophoresed into a gel identical to that of Fig. 1 separately and in combination (after brief mixing at 0°C for 10 S). Column 1: Untreated 16S rRNA (2 μg); column 2, untreated (1 μg) and neomycin-treated (1 μg) 16S rRNA; column 3, neomycin-treated 16S rRNA (2 μg).*

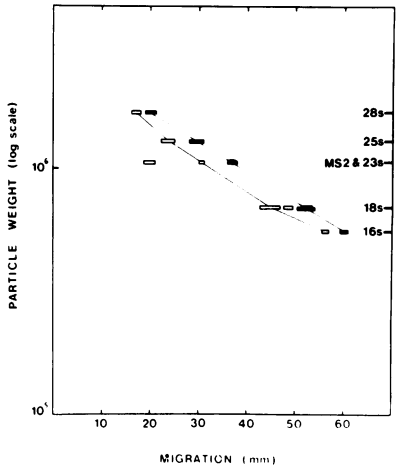


FIG. 9. *Relationship between the particle weight and the migration in the gel of control and neomycin-treated rRNAs. RNAs were isolated from E. coli (16S and 23S), yeast (18S and 25S), and rat liver (18S and 28S). Neomycin was added to portions of the RNAs as in Fig. 2, followed by electrophoresis of control and neomycin-treated samples into a 2.5% acrylamide–0.5% agarose gel, under conditions otherwise identical to those of the gel in Fig. 1.* (□) *Control RNA;* (■) *neomycin-treated RNA. Broader rectangles represent 18S RNA from yeast and MS2 RNA.*

thus such an increase in mobility must represent a reduced molecular size in comparison to that of the molecules not receiving neomycin. We propose that the basic amino groups on the neomycin molecule interact with negative charges in the RNA at distant sites allowing for a stabilization of the RNA structure (or ribosome) and restricting the unfolding of the molecules, which otherwise happens during gel electrophoresis in magnesium-deficient buffers. This effect is of sufficient magnitude to offset an increased particle weight introduced by the bound antibiotic. This proposal is supported by the data of Fig. 6. Ribosomes were stabilized by neomycin while in buffers with a higher magnesium ion concentration, and, accordingly, in a more compact structure, they migrated further into the gel than the more unfolded ribosomes at lower magnesium ion concentrations (Fig. 6). The increased sedimentation rates of neomycin-treated RNAs (Fig. 7) can be accounted for in part by both a rigid, less unfolded structure and an increased particle weight due to the bound neomycin. A very tightly packed structure was also proposed by Altescu (1) who used a much higher neomycin concentration than was used in this report to produce insoluble complexes resistant to pancreatic RNase.

A progressive increase in ribosome and rRNA mobility in acrylamide gels is noted with increasing neomycin concentrations indicating that neomycin binds at multiple sites on rRNA, as originally suggested by the functional studies of Davies and Davis (8). The added neomycin increasingly restricts the unfolding of the molecules. The binding is of sufficient stability to survive dialysis, but is in an apparent equilibrium as evidenced by the rapid conversion of two RNAs, with and without bound neomycin, to a single intermediate species upon mixing (Fig. 7). Presumably the more "weakly" bound neomycin molecules rapidly transfer to sites of greater affinity on the RNA not previously exposed to neomycin. The total number of binding sites on the 16S rRNA was not determined but assuming 100% binding, the minimal number required to achieve maximum increase in electrophoretic mobility was estimated by titration studies to be between about 100 and 150.

Paromomycin and ribostamycin, structurally very similar to neomycin, have significant differences in their effects on ribosome and rRNA mobility. The replacement of a single amino group in neomycin by an hydroxyl group in paromomycin at one end of the antibiotic molecule reduces by 50% the effect on ribosome and rRNA mobility. Ribostamycin, lacking the 6-amino-L-glucosamine group at the other end of the neomycin molecule, produces no change in rRNA mobility. Although these data demonstrate the importance of the amino groups in the neomycin molecule, they are not sufficient to permit one to assign precise functional roles to specific amino groups.

The extent to which different aminoglycoside antibiotics restrict the unfolding of the ribosome structure, as detected by increased gel electrophoretic mobility, correlates well with what is known about their capacities to induce misreading and inhibit translation. Gradual increases in ribosome mobility are observed with neomycin and gentamicin at low antibiotic concentrations where they also induce a gradual increase in misreading (8). A similar pattern is noted for paromomycin and kanamycin, which also induce misreading. However, at higher antibiotic concentrations, only neomycin (at 10^{-5} M) and gentamicin (at 10^{-4} M) produce the striking increase in ribosome mobility shown in Fig. 3. This occurs at the same concentration at which inhibition of translation is observed (8). Neither the striking increase in ribosome mobility nor the inhibition of translation is observed with paromomycin, kanamycin, or any of the other antibiotics tested. These data suggest that the ribosome (and the rRNA) may become increasingly restricted in movement of functionally important structural

components with increased binding of certain aminoglycoside antibiotics. Functionally, this may be expressed by a progressive increase in misreading. Inhibition of translation may occur when sufficient antibiotic has bound to the ribosome to totally restrict movement beyond a critical point. The neomycin binding of the 5S rRNA to the 50S subunit is an example.

The marked in vitro effect of neomycin on MS2 RNA gel electrophoretic mobility suggests that interactions of this type in which neomycin restricts the unfolding of mRNA during translation, might also contribute to the efficiency of neomycin as an antibiotic.

The gel electrophoresis method does not provide any evidence that neomycin introduces any structural limitations on the smaller RNAs such as tRNA's. These are tightly folded molecules which both neomycin and paromomycin appear to influence equally by increasing the particle weight and retarding electrophoretic mobility.

Neomycin has proved to be beneficial in providing a more accurate estimation of RNA molecular weight by the acrylamide gel electrophoresis method. The procedure is limited to large RNAs and, as might be expected, the precision is not equivalent to that obtained with formamide gels (13). However, it is a simple, rapid technique with the added advantage that it permits comparison of the treated and untreated samples in adjacent slots of the same gel.

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APPENDIX

Circular Dichroism Study of the Effect of Neomycin on Ribosomes

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Circular dichroism (CD) spectroscopy is capable of yielding structural information on optically active biological macromolecules (4). Nucleic acids in particular have CD spectral properties that can be interpreted in terms of both secondary structure (base pairing and base stacking) and tertiary structure (overall conformation). To assess the nature of the effect produced by neomycin on *E. coli* ribosomes, as described in the accompanying paper by Dahlberg et al., the CD spectra shown in Fig. 1 have been obtained. The spectra show the characteristic positive (265-nm) and negative (295-nm) bands expected for solutions containing polymeric RNA (5). The spectra were not extended to lower wavelengths because the presence of ultraviolet-absorbing EDTA in some experiments prevented such measurements. In the presence of 2×10^{-4} M neomycin (150 μg/ml) the 70S ribosome spectrum is unchanged.

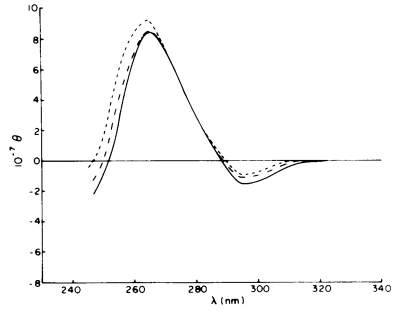

FIG. 1. *CD spectra of 70S ribosomes in 150 mM NH_4Cl–50 mM Tris-hydrochloride (pH 7.5)–10 mM $Mg(OAc)_2$. The spectra were recorded in 1-cm pathlength cells at an ambient temperature on a Cary 61 spectropolarimeter. The results are calculated in terms of molar ellipticity in terms of 70S ribosomes as described earlier (5). The ribosome concentration is estimated by using a molecular weight of 2.6×10^6 and an absorbance at 260 nm of 15 for 1 mg/ml. (———) 70S ribosome in the presence and absence of 2×10^{-4} M neomycin. (– – –) 70S ribosome in the presence of 50 mM EDTA. (· — · — · — ·) 70S ribosome in the presence of 50 mM EDTA and 2×10^{-4} M neomycin.*

Consequently, there must be no substantial alteration in the RNA secondary or tertiary structure when the drug binds to its receptor site(s) on the ribosome, consistent with the conclusion drawn above by Dahlberg et al. (1) from polyacrylamide gel electrophoresis studies.

The presence of 50 mM EDTA produces three distinct changes in the CD spectrum of 70S ribosomes. These changes arise from the fact that EDTA forms complexes with essential Mg^{2+} ions and causes the ribosome to unfold (3). First, the weak negative band at 295 nm has a reduced intensity in the unfolded ribosome. The exact nature of this transition is not well understood, but its presence is believed to be associated with the formation of base-paired conformations (4, 5). Hence, as might be expected, EDTA-induced unfolding reduces the extent of double-helical secondary structure. In addition, the intensity of the 265-nm band is increased in the EDTA-treated ribosome. This increase in ellipticity is generally ascribed to an increase in another aspect of secondary structure, namely base stacking (2). Thus, the EDTA-unfolded 70S particle tends toward a structure containing less base pairing and more base stacking. Still a third spectral change in the EDTA-treated ribosome is a broadening of the transition below 265 nm; this is probably also related to a loss of base-paired regions.

The CD spectra in Fig. 1 also reveal that the presence of neomycin protects the ribosome from the unfolding caused by EDTA. The reduction in the negative intensity of the 295-nm band is not as pronounced in the presence of the drug, indicating that less of the base-paired structure is lost upon unfolding. In addition, the increase in the 265-nm band characteristic of EDTA-induced unfolding is not observed when both neomycin and EDTA are in solution with the 70S ribosomes. Last, the low-wavelength broadening is intermediate between native and EDTA-unfolded ribosomes when the drug is present. Thus, the optical properties are fully consistent with the notion derived by Dahlberg et al. (1) that bound neomycin restricts the unfolding of the *E. coli* ribosome.

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