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INDUCTION OF TRANSLATIONAL ERRORS (MISREADING) BY TUBERACTINOMYCINS AND CAPREOMYCINS

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SUMMARY. The well characterized translocation inhibitor viomycin (=tuberactinomycin B) promotes translational errors (misreading) in an *in vitro* system from *Escherichia coli*. It strongly stimulates both the binding of noncognate Tyr-tRNA to poly(U)-programmed ribosomes and the subsequent synthesis of AcPhe$(Tyr)_{\bar{n}}$-tRNA ($\bar{n}\simeq 20$). The closely related antibiotics capreomycin and tuberactinomycins A,N and O also inhibit translocation and induce misreading.

INTRODUCTION. The tuberactinomycin and capreomycin groups of antibiotics comprise several closely related basic peptides that interfere with protein synthesis in bacterial systems (1,review). Their mechanism of action has been examined in some depth only in the case of viomycin (=tuberactinomycin B). This antibiotic binds to both ribosomal subunits and interferes with the elongation cycle of protein synthesis by blocking the exit of peptidyl-tRNA from the ribosomal acceptor (A) site, thereby inhibiting translocation (2-4). In addition, viomycin inhibits initiation of polypeptide synthesis by preventing the binding of fMet-tRNA to the ribosomal 30S subunit (3).

It has recently been shown that the aminoglycoside antibiotics of the neomycin, kanamycin and gentamicin groups, as well as hygromycin B, also inhibit translocation with a mechanism very similar to that of the chemically unrelated viomycin (5,6). Moreover, several of these aminoglycosides (and also viomycin) compete with tuberactinomycin O for binding to both ribosomal subunits (2). Further similarities between the mechanism of action of the aminoglycosides and the tuberactinomycins and capreomycins now become apparent in that the viomycin-like antibiotics induce errors in the translation of the genetic message (misreading). This is also a well known property of the aminoglycoside antibiotics of the groups indicated (1).

METHODS. Preparation of 1 M NH_4Cl-washed *E. coli* MRE600 ribosomes, EF-G, EF-T (EF-Ts+EF-Tu), and S100 extracts (supernatants from a 100.000×g spin) depleted of endogenous tRNA by DEAE-cellulose chromatography has been described elsewhere (7-10). Ac[^{14}C]Phe-tRNA (970 cpm/pmol) and [^{3}H]Tyr-$tRNA^{Tyr}$ (1600 cpm/pmol) were prepared from purified $tRNA^{Phe}$ and $tRNA^{Tyr}$, respectively (Sigma, St.Louis, USA). Viomycin was a gift from Dr. H.E.Machamer, Parke-Davis

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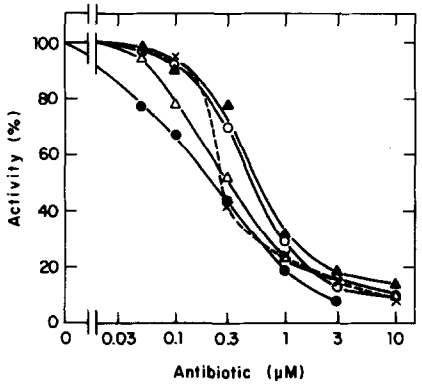


Fig. 1. Inhibition by viomycin (O), capreomycin (×) and tuberactinomycin A (Δ), N (▲) and O (●) of the EF-G plus GTP-dependent synthesis of Ac[^{14}C]Phe-puromycin. Translocation of Ac[^{14}C]Phe-tRNA and puromycin reaction was carried out in mixtures (20 μl) containing : 50 mM NH_4Cl, 15 mM KCl, 10 mM magnesium acetate, 18 mM Tris-HCl pH 7.8, 1 mM dithiothreitol, 6-7 pmol of ribosomes containing 2.9-3.7 pmol of A-site-bound Ac[^{14}C]Phe-tRNA, 70 μg/ml EF-G, 0.15 mM GTP, 0.5 mM puromycin and antibiotic as specified. The reaction was started by the addition of EF-G plus GTP and heating the tubes to 30°. After 3 min of incubation at this temperature, the mixtures were analyzed for Ac[^{14}C]Phe-puromycin synthesized (13). Values from controls without EF-G and GTP were subtracted. One hundred percent represents 1.2 pmol of Ac[^{14}C]Phe-puromycin synthesized.

Co., Detroit, USA, and tuberactinomycins A, N and O from Dr. Toshiharu Noda, Toyo Jozo Co., Japan. Capreomycin sulfate (a mixture of capreomycins IA, IB, IIA and IIB) was a gift from Lilly Indiana of Spain, Madrid.

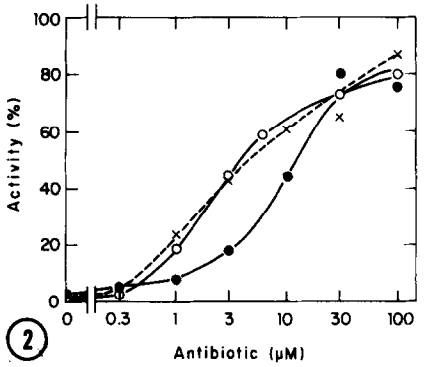
Ribosomes were activated in the presence of poly(U) prior to each experiment (11,12). Nonenzymic binding of Ac[^{14}C]Phe-tRNA to the ribosomal P-site was carried out at 8 mM magnesium acetate (12), while that to the ribosomal A-site was conducted in the presence of 20 mM magnesium acetate and deacylated $tRNA^{Phe}$ (11). Other methods are specified in the legends to the figures.

RESULTS. *Inhibition of translocation by tuberactinomycins and capreomycins.* Since very little is known of the mechanism of action of capreomycins and tuberactinomycins other than viomycin, we have investigated whether these little studied antibiotics are, like viomycin, inhibitors of translocation. Figure 1 shows the effect of increasing concentrations of tuberactinomycins A, N and O and of a mixture of capreomycins on the EF-G plus GTP-dependent reaction of ribosome-bound Ac[^{14}C]Phe-tRNA with puromycin. All the antibiotics effectively inhibited the reaction at concentrations higher than 3 μM. At lower concentrations, tuberactinomycins A and O, and possibly capreomycin, seemed rather more inhibitory than viomycin, while tuberactinomycin N was equally as effective as viomycin. These antibiotics did not significantly interfere with the peptidyl transferase reaction since they did not modify the reaction of P-site-bound Ac[^{14}C]Phe-tRNA with puromycin (not shown).

Stimulation of the binding of Tyr-tRNA to poly(U)-programmed ribosomes. The binding of a noncognate aminoacyl-tRNA, in this case Tyr-tRNA^{Tyr}, to poly(U)-programmed ribosomes is a convenient assay to detect misreading induced by antibiotics. The binding of Tyr-tRNA^{Tyr} requires EF-T and GTP and takes place at the ribosomal A-site (14). To make comparisons between the different experiments possible, a control mixture with 3 μM neomycin B (a powerful inducer of misreading (1,14)) was included in each experiment and the results have been expressed as percentages of the binding obtained with this control antibiotic. Figure 2 shows that both viomycin and capreomycin already stimulated binding of Tyr-tRNA at concentrations lower than 1 μM, but 30 to 100 μM were needed to saturate binding. Under these latter conditions, 80% as much Tyr-tRNA was bound as with neomycin B. Tuberactinomycin O, at low concentrations, was less active than viomycin and capreomycin, but it was equally effective at high concentrations (Fig. 2).

Synthesis of AcPhe(Tyr)$_n$-tRNA. The above experiments demonstrated that tuberactinomycins and capreomycins could promote binding of a noncognate aminoacyl-tRNA to the ribosome. Consequently, it was felt desirable to investigate whether Tyr-tRNA was bound correctly into the ribosomal A-site and, therefore, whether its tyrosyl residue could be incorporated into a peptidyl nascent chain. Accordingly, ribosomes were complexed with poly(U) and Ac[^{14}C]Phe-tRNA in the P-site and were supplemented with an S100 extract containing tRNA^{Tyr}, [^{3}H]tyrosine and the remaining compounds necessary for *in vitro* polypeptide synthesis. Figure 3 shows that, in this system, each tuberactinomycin or the mixture of capreomycins readily promoted the incorporation of [^{3}H]tyrosine into hot-trichloroacetic acid precipitable material. Since the reaction required the presence of Ac[^{14}C]Phe-tRNA on the ribosomes (not shown), and part of the ^{14}C-radioactivity present also became precipitable in that acid, it seems reasonable to assume that the material synthesized was Ac[^{14}C]Phe([^{3}H]Tyr)$_n$-tRNA. Under optimal conditions (100 μM antibiotic) the number of tyrosyl residues incorporated in each polypeptide chain reached an average of 20 (legend to Fig. 3). Thus, the tuberactinomycins and capreomycins induce strong misreading in this *in vitro* system.

DISCUSSION. Tuberactinomycins and capreomycins promote misreading in an *in vitro* polypeptide synthesizing system that contains Tyr-tRNA^{Tyr} as the sole species of free aminoacyl-tRNA (Fig. 3). When this system is supplemented with the cognate Phe-tRNA^{Phe} and other aminoacyl-tRNA's, very little misreading is observed at either high (100 μM) or low (*ca.* 1 μM) concentrations of the antibiotics (our unpublished results). A strong inhibition of transloca-



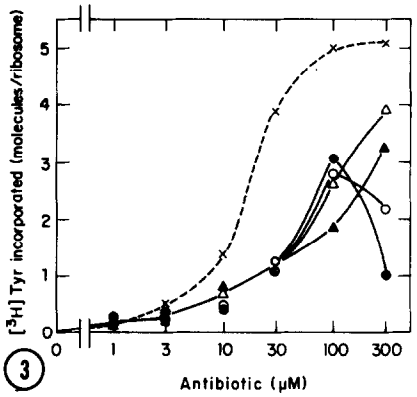


Fig. 2. Stimulation by viomycin (O), tuberactinomycin O (●) and capreomycin (×) of the binding of [^{3}H]Tyr-tRNA to poly(U)-programmed ribosomes. Binding was performed in mixtures (20 μl) containing: 65 mM NH_4Cl, 10 mM magnesium acetate, 20 mM Tris-HCl pH 7.5, 1 mM dithiothreitol, 6 pmol poly(U)-programmed ribosomes, 0.15 mM GTP, 0.3 μM $tRNA^{Phe}$, 0.5 μM [^{3}H]Tyr-tRNA, 12 μg/ml EF-T and antibiotic as specified. Binding was started by addition of [^{3}H]Tyr-tRNA. Incubation at 30° for 20 min was followed by determination of the amount of bound [^{3}H]Tyr-tRNA by the nitrocellulose filter technique (15). Values of parallel mixtures without poly(U) have been subtracted. One hundred percent represents the amount of [^{3}H]Tyr-tRNA bound in control mixtures with the indicated antibiotics replaced by 3 μM neomycin B (2.1-5.6 pmol).

Fig. 3. Stimulation by viomycin (O), capreomycin (×) and tuberactinomycins A (Δ), N (▲) and O (●) of the synthesis of Ac[^{14}C]Phe-([^{3}H]Tyr)$_n$-tRNA. Noncognate peptidyl-tRNA synthesis was performed in mixtures (25 μl) containing: 35 mM NH_4Cl, 25 mM KCl, 12 mM magnesium acetate, 50 mM Tris-HCl pH 7.8, 1 mM dithiothreitol, 7 pmol of ribosomes complexed with poly(U) and P-site-bound Ac[^{14}C]Phe-tRNA, 2 μM $tRNA^{Tyr}$, 10 μM [^{3}H]tyrosine (820-1150 cpm/pmol), 5 mM phosphoenol pyruvate, 60 μg/ml pyruvate kinase, 2 mM ATP, 0.25 mM GTP, a saturating amount of S100 extract (0.7 mg/ml) and antibiotic as indicated. The reaction was started by addition of the S100 extract and by heating the tubes to 30°. After 25 min of incubation at this temperature, the mixtures were analyzed for ^{14}C and ^{3}H radioactivity precipitable in hot trichloroacetic acid. The ratio of incorporated [^{3}H]Tyr plus Ac[^{14}C]Phe over Ac[^{14}C]Phe yields the average length in number of amino acid residues of the peptides synthesized. This ranged, for the reactions showing maximum [^{3}H]Tyr incorporation with each antibiotic, between 16 and 21 amino acid residues per chain. Data for capreomycin are not strictly comparable with those for the tuberactinomycins since they were obtained in a different experiment.

tion seems to be the prevalent effect. This result agrees with a previous report in which, with a complete polypeptide synthesizing system directed by synthetic homopolynucleotides, viomycin failed to induce detectable misreading (16). The explanation for the apparently different behaviour of the antibiotics in the two systems resides in our finding that the noncognate peptidyl-$tRNA^{Tyr}$ (and presumably other noncognate peptidyl-tRNA's) is loosely held on the ribosome (12). Viomycin does not block peptidyl-$tRNA^{Tyr}$ on the ribosomal A-site, and multiple rounds of binding of Tyr-$tRNA^{Tyr}$ and elongation of the peptidyl chain can take place. In a system containing all aminoacyl-tRNA's,

each time the correct Phe-tRNA^{Phe} is bound, the subsequently synthetized peptidyl-tRNA^{Phe} has a high probability of being trapped in the A-site by the action of the antibiotics; synthesis then stops and misreading cannot be expressed. It thus seems likely that the inhibition of translocation (and/or initiation) may be the prevalent effect of the drugs *in vivo*. Nevertheless, our results clearly show that, similar to many aminoglycosides, the tuberactinomycins and capreomycins have the potential of disturbing the process of aminoacyl-tRNA selection. It would be interesting to attempt the detection of phenotypic suppresion of appropriate mutations in an organism permeable to these antibiotics (1).

At present, it is not known whether the same or different interaction(s) of the tuberactinomycins and capreomycins with the ribosome are responsible for the interference with translocation and aminoacyl-tRNA selection. In fact, the difference in the concentrations of antibiotic required for each effect (compare Fig. 1 with Figs. 2 and 3) suggests that different interactions may be responsible. However, previous work from our laboratory has indicated that binding of neomycin B to the ribosomal 30S subunit promotes misreading and also impairs translocation (17). Since neomycin B and the tuberactinomycins and capreomycins seem to have very similar mechanisms of action and closely related ribosomal binding sites (1,3-6,18), it is tempting to suggest that the interaction of the peptide antibiotics with the 30S subunit may also affect translocation and aminoacyl-tRNA selection. Other work has already indicated a strong relationship between these processes (reviewed in 5,19).

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