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BINDING OF [^{14}C]TUBERACTINOMYCIN O, AN ANTIBIOTIC CLOSELY RELATED TO VIOMYCIN, TO THE BACTERIAL RIBOSOME

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SUMMARY

The binding of [^{14}C]tuberactinomycin O, an antibiotic closely related to viomycin, to E. coli ribosomes has been examined by equilibrium dialysis method. The antibiotic has been observed to bind to the 70S ribosome, which possesses two binding sites: one on the 30S ribosomal subunit and another on the 50S subunit. The affinity for the large subunit is greater than that for the small subunit. The binding to both ribosomal subunits is reversed by viomycin, indicating that tuberactinomycin O and viomycin have the same binding sites on the ribosome. The results seem to be in accordance with the previous finding that viomycin exhibits dual actions on ribosomal function: the inhibition of fMet-tRNA$_F$ (initiation) and inhibition of translocation of peptidyl-tRNA.

Viomycin, a basic peptide antibiotic, is a potent inhibitor of protein synthesis (1-3). It has been observed that the antibiotic exhibits dual actions on the ribosomal functions, blocking initiation and elongation process of protein synthesis (3). The analysis of these effects has revealed that viomycin prevents the binding of fMet-tRNA$_F$ to the 30S ribosomal subunit and the translocation of peptidyl-tRNA from the acceptor site to the donor site on the ribosome, while it does not significantly affect aminoacyl-tRNA binding or the peptidyl transferase reaction (3). The inhibition by the antibiotic of peptidyl-tRNA translocation has been also demonstrated in a salt-washed polysomal system (4).

The interference of viomycin with the 30S initiation complex formation indicates that the antibiotic acts on the 30S ribosomal subunit (3). However, it remains to be determined whether the inhibition of translocation of peptidyl-tRNA is caused by the interaction with the 50S ribosomal subunit or with the 30S subunit. For the purpose of elucidating this problem, [^{14}C]tuberactinomycin O, an antibiotic closely related to viomycin (Fig. 1), has been prepared, and the binding to Escherichia coli ribosomes has been studied. Instead of viomycin, tuberactinomycin O has been employed for radioactive labelling, because of the stability in the process of chemical modification (5,6). The resuts are presented in this communication.

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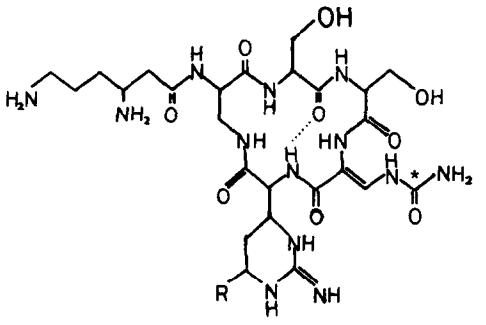


Fig. 1. Chemical structure of viomycin and tuberactinomycin O.
Viomycin: R = OH, Tuberactinomycin O: R = H.
* The asterisk represents the position, labelled with radioactive carbon atom.

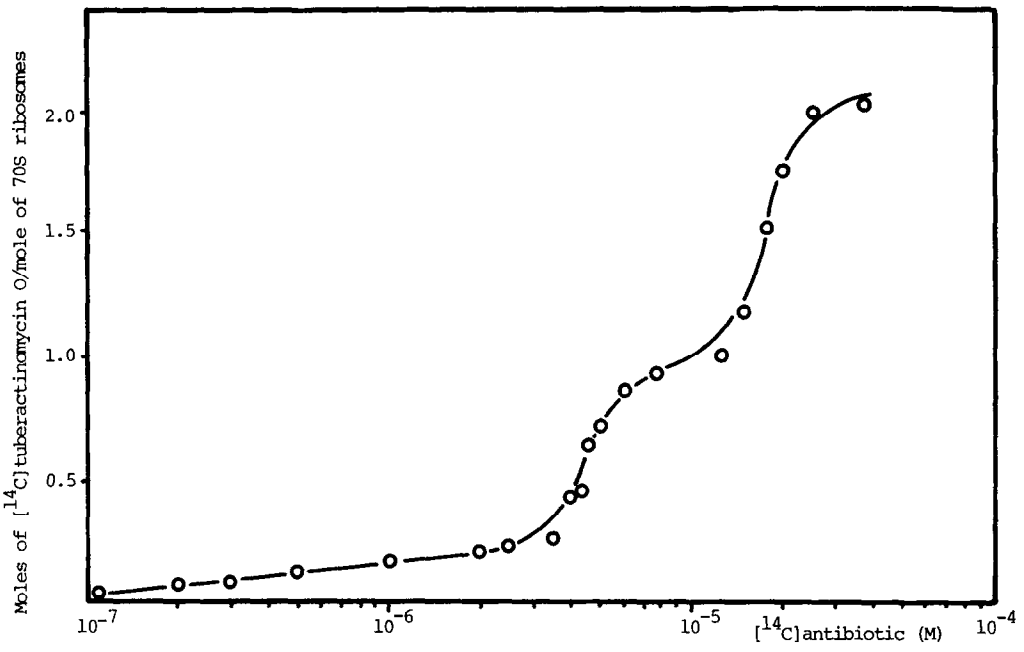
MATERIALS AND METHODS

[^{14}C]Tuberactinomycin O was prepared by incubating 15 mg of the antibiotic with the same amount of [^{14}C]urea (a product of Radiochemical Centre, Amersham, England) in 0.3 ml of 3 N HCl for 40 days at room temperature, and purified by Sephadex G10 column chromatography. [^{14}C]Tuberactinomycin O, thus obtained, gave a single spot by paper chromatography, using a solvent system (acetone : 10% ammonium acetate : 10% ammonia water = 30 : 9 : 1); and it showed a specific activity of 15.0 Ci/mole (Fig. 1).

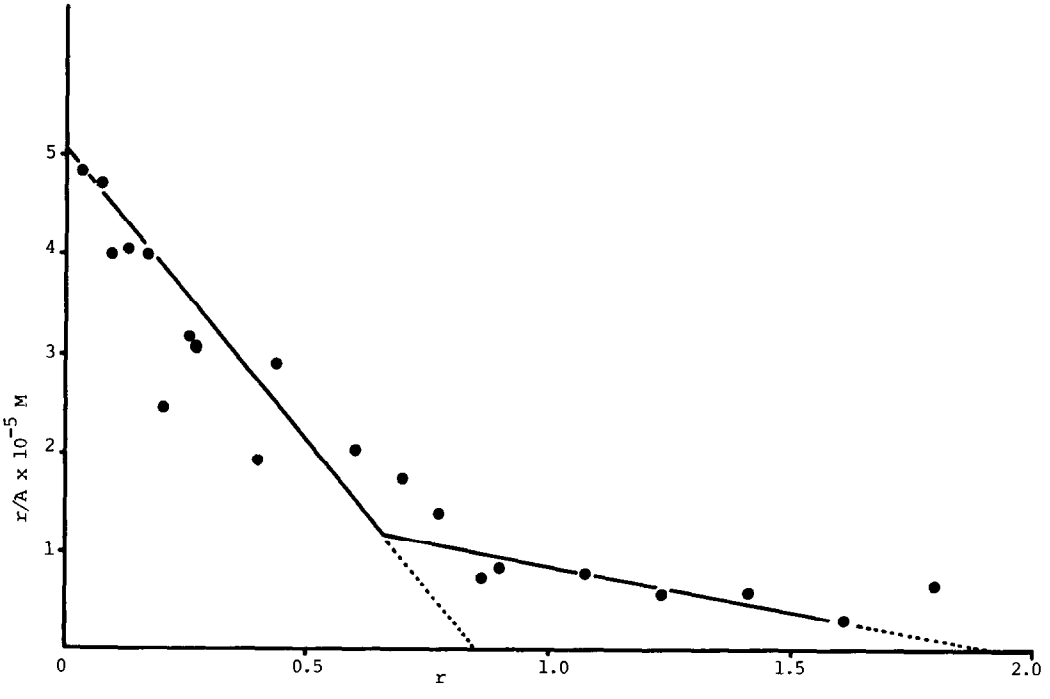
Escherichia coli Q13 grown to mid-logarithmic phase of growth was used; and the ribosome and ribosomal subunits were prepared by the method of Staehelin and Maglott (7). The interaction of [^{14}C]tuberactinomycin O with the 70S ribosome or ribosomal subunit was measured by equilibrium dialysis technique. The ribosome or ribosomal subunit (each 100 pmoles), suspended in 0.1 ml of a standard buffer [Tris-HCl, pH 7.5, 10 mM, NH_4Cl 80 mM, $Mg(AcO)_2$ 10 mM, and 2-mercaptoethanol 6 mM], was placed in a chamber; and various concentrations of [^{14}C]tuberactinomycin O in 0.1 ml of the same buffer in the other chamber. Both chambers were separated by Visking cellulose tubing membrane. The apparatus was kept at 4°C for 18 - 24 hours with gentle shaking. The period was long enough for equilibration. The radioactivity in both chambers was determined in a liquid scintillation counter. The difference was taken to represent the amount of bound tuberactinomycin O.

RESULTS

[^{14}C]Tuberactinomycin O was demonstrated to bind with the ribosomes. For the purpose of measuring the association constants and number of binding sites on the 70S ribosome, the equilibrium dislysis experiment was performed over a range of concentrations of [^{14}C]tuberactinomycin O. It was found that, at saturating levels of the antibiotic, approximately two moles of the drug were bound per mole of the ribosome (Fig. 2). The shape of the binding curve also suggested that there might be at least two binding sites with different association constants. By the method employed, approximately one-fifth of the antibiotic



ig. 2. Effect of concentration of [^{14}C]tuberactinomycin O on binding to 70S ribosomes.



g. 3. Scatchard plot for equilibrium binding of [^{14}C]tuberactinomycin O to 70S ribosomes

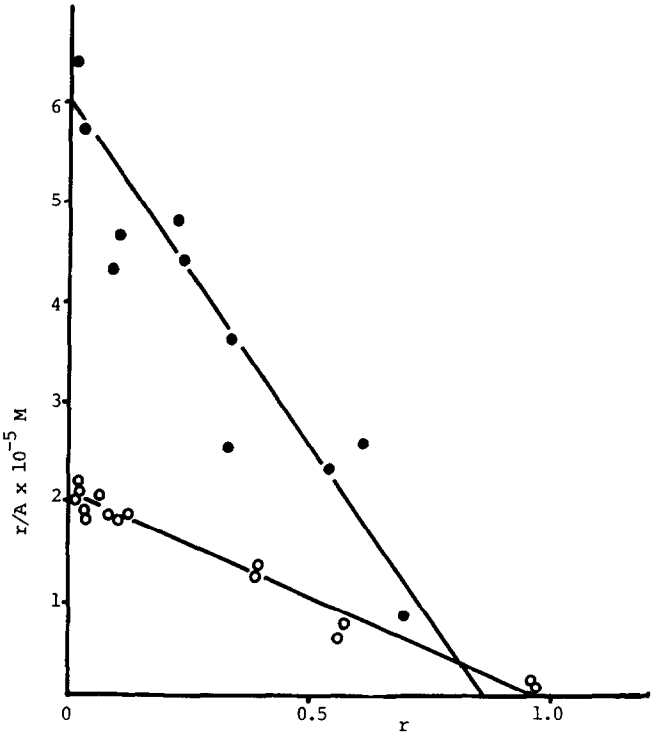


Fig. 4. Scatchard plot for equilibrium binding of [^{14}C] tuberactinomycin O to the ribosomal subunits.
—o—o— 30S subunit —●—●— 50S subunit

was bound to the ribosome at the concentration of one μM, representing equal molar ratio to ribosomes.

Figures 3 and 4 show Scatchard plots (8) of data for the equilibrium binding of the antibiotic to the ribosome and ribosomal subunits. There appeared to exist a linear relationship between r and r/A, where r represents moles of bound [^{14}C]tuberactinomycin O per mole of ribosomes or subunits, and A represents molar concentration of the free [^{14}C]antibiotic. The 70S ribosome seemed to possess one binding site with an association constant of approximately 6.0 x 10^5 M^{-1}, and another site with a lower binding constant. The number of binding sites on the 30S ribosomal subunit was one (r = 0.95) with an association constant of 2.3 x 10^5 M^{-1}, and that of the 50S subunit was one (r = 0.85) with a binding constant of 7.2 x 10^5 M^{-1}. The association constant was estimated from the slope of Scatchard plots. Thus, each ribosomal subunit possesses one binding site for tuberactinomycin O, and the large subunit shows higher affinity than the small subunit.

Table 1. Effects of antibiotics on the binding of [^{14}C]tuberactinomycin O to the ribosome and ribosomal subunits.

Antibiotics	Ribosomes	Ribosomal subunits	
	70S	50S	30S
None	89	79	58
Tuberactinomycin O	12	12	6.4
Viomycin	10	10	5.3
Streptomycin	64	71	40
Erythromycin	85	72	54

The number represents pmoles of [^{14}C]tuberactinomycin O bound to 100 pmoles of the ribosome or ribosomal subunit.

The equilibrium dialysis was carried out as described in MATERIALS AND METHODS. The dialysis mixture, in 0.1 ml of the buffer, contained *E. coli* ribosomes or ribosomal subunits 1 μM, [^{14}C]tuberactinomycin O 10 μM and cold antibiotics 100 μM.

Effects of some antibiotics on the interaction of [^{14}C]tuberactinomycin O was studied by the equilibrium dialysis method; and the results are summarized in Table 1. The binding of radioactive material to the 70S ribosome and ribosomal subunits was diluted by the presence of cold tuberactinomycin O, indicating that the observed binding was specific for the antibiotic.

Viomycin was observed to reverse the binding of [^{14}C]tuberactinomycin O to the ribosome and both ribosomal subunits at the same levels with cold tuberactinomycin O. The radioactive binding was not significantly affected by erythromycin. Streptomycin seemed to decrease somewhat the binding to the 30S ribosomal subunit but not that to the 50S subunit (Table 1).

DISCUSSION

It has been observed by the equilibrium dialysis procedure that tuberactinomycin O possesses two binding sites on the 70S ribosome: one on the 30S ribosomal subunit and another on the 50S subunit. Reversible binding of the antibiotic has been also demonstrated by a Millipore filter method. The binding is dependent upon temperature: for example, the antibiotic binds to the 70S ribosome in a molar ratio of 0.2 : 1 at 4°C (Fig. 2) and 0.5 : 1 at 37°C at an antibiotic concentration of one μM, which causes approximately 50% inhibition of polyphenylalanine synthesis (data are not shown). It remains to be determined whether a single molecule of the antibiotic binds to both ribosomal subunits or separate molecules bind to each subunit.

The binding of the [^{14}C]antibiotic is reversed by viomycin at the same levels as with cold tuberactinomycin O. Since these antibiotics have similar chemical

structures, the antagonism may be due to competition for the same binding sites on the ribosome. The dual effects of viomycin on the ribosomal functions previously reported (3), are supported by the above results. The affinity of the 50S ribosomal subunit is greater than that of the 30S subunit; this appears to be in accordance with the observation that viomycin blocks translocation of peptidyl-tRNA at lower concentration than fMet-tRNA$_F$ binding to the small ribosomal subunit (3). The binding of the antibiotic to the large ribosomal subunit seems to result in the inhibition of translocation of peptidyl-tRNA from the acceptor site to the donor site. However, there still remains a possibility that the inhibition of peptidyl-tRNA translocation might be caused by the interaction with the small ribosomal subunit. Martinez et al. (9) have demonstrated that the binding of streptomycin or viomycin to E. coli ribosomes modifies the iodination of at least 5 or 9 ribosomal proteins, respectively. These include proteins of large and small subunits, suggesting that streptomycin as well as viomycin may interact with both ribosomal subunits. However, the interpretation of the results may not be simple.

Tuberactinomycin O and viomycin possess several functional groups in the molecule. It remains to be determined which functional group(s) is important for the interaction with the ribosome and whether the same functional group(s) bind(s) to both ribosomal subunits. We are also attempting to determine which components of the ribosome are essential for the binding of viomycin or tuberactinomycin O.

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