BINDING OF [14C]TUBERACTINOMYCIN O TO RIBOSOMES FROM MYCOBACTERIUM SMEGMATIS

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The peptide antibiotics, tuberactinomycin O (Tum O) and viomycin (=tuberactinomycin B, VM) are closely related in chemical structure; both are more active against mycobacteria than other bacterial species. The mode of action of VM has been studied in detail; VM inhibits protein synthesis in Mycobacterium avian and in cell-free systems derived from Escherichia coli and Mycobacterium smegmatis. It is a ribosomal inhibitor because resistance to VM in mutants of M. smegmatis is due to ribosome alterations. Some of the mutants contained altered 50S subunits, whereas others had altered 30S subunits. Genetic and biochemical studies have confirmed that there are two loci for VM resistance on the chromosome of M. smegmatis. VM was a product of Pfizer Taito Co., Ltd. (Japan). Tum O was a gift from Toyo Jozo Co., Ltd. (Japan). [14C]Tum O was prepared according to the procedures described previously.

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TMXNYSH (10 mm Tris-HCl, pH 7.8, 5 mm Mg-acetate, Y mm NH₄Cl, 6 mm 2-mercaptoethanol) were used. TMNSH: T1OM1ON60SH buffer. The preparation of ribosomes and the cell-free system for poly U-directed polyphenylalanine synthesis were as described previously with a few modifications. The standard reaction mixture (0.1 ml) contained the following: 95 mm Tris-HCl, pH 7.8; 5.8 mm 2-mercaptoethanol; 7.5 mm phosphoenolpyruvate trisodium salt; 0.9 mm adenosine triphosphate; 0.028 mm guanosine triphosphate; 0.028 mm guanosine triphosphate; 0.1 mm [14C]phenylalanine (specific activity, 100 μCi/5.5 μmol); 3 A260 units of ribosomes, 100 μg of polyU; 50 μg of E. coli transfer RNA; and 10 mM magnesium acetate. The binding of [14C]Tubercactinomycin O binds more strongly to 70S ribosomes from M. smegmatis than those from E. coli and a drug-resistant mutant of M. smegmatis. 2) The agent had a higher affinity for 70S ribosomes than either ribosomal subunit derived from M. smegmatis. M. smegmatis 70S ribosomes seem to carry the binding sites of the antibiotic important for inhibition of polypeptide synthesis.

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Table 1. Binding of \([^{14}C]\text{Tum O}^a\)

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Ribosome</th>
<th>pmol of ([^{14}C]\text{Tum O} \text{ per pmol of ribosomes})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(M. \text{smegmatis})</td>
<td>70S</td>
<td>0.22$\pm$0.48 2.6</td>
</tr>
<tr>
<td>(\text{R-15})</td>
<td>50S</td>
<td>$&lt;0.045$ 2.6</td>
</tr>
<tr>
<td></td>
<td>30S</td>
<td>$&lt;0.05$ 2.6</td>
</tr>
<tr>
<td>(M. \text{bovis BCG})</td>
<td>70S</td>
<td>ND$^c$ 2.8</td>
</tr>
<tr>
<td>(M. \text{smegmatis})</td>
<td>70S</td>
<td>0.1$\pm$0.06 3.0</td>
</tr>
<tr>
<td>(\text{VM-resistant})</td>
<td>70S</td>
<td>0.07$\pm$0.08 2.2</td>
</tr>
</tbody>
</table>

\(a\) Experimental procedures are described in the text.

\(b\) The binding was examined by means of dialysis against excess amounts of buffer. The values show the variation of at least 3 separate estimates.

\(c\) For equilibrium dialysis, one chamber contained 180 pmol of 70S and the other 2 nmol of \([^{14}C]\text{Tum O}\) in 100 \(\mu\)l of TMNSH buffer. When equilibrium was reached after 20 hours, aliquots were removed and counted in a scintillation counter.

\(d\) Not done.

were determined. Amount (pmol) of ribosomes were calculated assuming that one A\(_{260}\) unit of 70S, 50S and 30S ribosomes corresponds to 24, 36 and 64 pmol respectively.

The results obtained by equilibrium dialysis suggest that the drug binds to ribosomes derived from \(M. \text{smegmatis}\), a VM-resistant mutant of \(M. \text{smegmatis}\), \(M. \text{bovis BCG}\) and \(E. \text{coli}\) (Table 1). No significant differences in the binding affinities were observed in these four cases. This observation is consistent with our previous report\(^{12}\).

On the contrary, binding studies by means of extensive dialysis indicated that the Tum O bound to ribosomes from \(E. \text{coli}\) and the VM-resistant mutant of \(M. \text{smegmatis}\) can be easily dissociated, whereas it binds strongly to ribosomes of drug-sensitive \(M. \text{smegmatis}\) ribosomes.

Whether the difference seen between the two dialysis methods represents simply differences in affinities, or a different type of interaction for the sensitive ribosomes is not clear.

The pattern of inhibition of poly U-directed polyphenylalanine synthesis by various concentrations of VM is presented in Fig. 1. As expected, VM has a greater effect on ribosomes from \(M. \text{smegmatis}\) and \(M. \text{bovis BCG}\) than on those from \(E. \text{coli}\) and the drug-resistant mutant of \(M. \text{smegmatis}\).

As shown in Table 1, the drug bound to either 50S or 30S subunits from \(M. \text{smegmatis}\) can be easily removed. This observation suggests that the antibiotic has strong affinity to 70S ribosomes very weak affinity to isolated ribosomal subunits.

This was confirmed by the following experiments. Excess amounts of either 50S or 30S subunits were added to a poly U-directed polyphenylalanine synthesizing system containing 70S ribosomes from \(M. \text{smegmatis}\) R-15, and the inhibitory effects of VM at various concentrations were examined. If the added subunits were capable of binding the drug, they should reduce the antibiotic-induced inhibition of polypeptide synthesis. The experimental results are presented in Figs. 2 and 3. As expected, neither subunit had any effect in restoring polypeptide synthesis in the presence of a variety of concentrations of VM. The addition of more 70S ribosomes reduced the extent of the inhibition.

Thus, we suggest that 70S ribosomes possess unique binding site(s) for the inhibitory effects of VM. Detailed analyses of the binding of Tum O to mycobacterial 70S ribosomes rather
Fig. 2. Inhibitory effect of VM on cell-free poly-peptide synthesis.

The cell-free system contained a 4-fold excess of 30S subunits to 70S ribosomes (○). The pattern of the inhibition of poly U-directed polyphenylalanine synthesis by VM on 70S ribosomes alone is shown as control (●). Experimental conditions are described in text. The incorporation of \[^{14}C\]phenylalanine into acid-insoluble material in the respective system in the absence of VM represents 100\% activity.

than to ribosomal subunits might be an important approach to an understanding of both the mode of action of VM and the complexity of ribosomal structure and function.

References


Fig. 3. Inhibitory effects of VM on cell-free polyphenylalanine synthesis.

The system containing a 4-fold excess 50S subunits to 70S ribosomes (○). The pattern of inhibition by VM on 70S alone is shown as control (●). The other conditions are as described in Fig. 2.

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