7-Hydroxytropolone: an Inhibitor of Aminoglycoside-2"-O-Adenylyltransferase

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Received 29 April 1982/Accepted 25 August 1982

Aminoglycoside-2"-O-adenylyltransferase was inhibited by 7-hydroxytropolone. Inhibition was competitive with respect to the cosubstrate ATP and appeared to require the unique vicinal arrangement of oxygens found in 7-hydroxytropolone. Combinations of 7-hydroxytropolone plus the appropriate aminoglycoside substrates were active against resistant bacteria possessing the adenylyltransferase. No potentiation was observed against other aminoglycoside-resistant or -susceptible strains. The fact that the inhibition of an aminoglycoside-modifying enzyme overcomes the poor uptake of aminoglycosides in resistant strains points to the singular importance of the inactivating enzyme as a determinant of resistance.

Resistance to aminoglycoside antibiotics in clinical isolates is frequently due to enzymatic modification of the antibiotic. The enzymes that modify aminoglycosides fall into three classes: N-acetyltransferases, O-adenylyltransferases, and O-phosphotransferases. These enzymes are genetically determined by transferable plasmids, some of which are known to be widespread in nature. The treatment of serious gram-negative infections by pathogens possessing one or more of these enzymes requires either the use of an aminoglycoside that does not serve as a substrate for the enzyme(s) or the use of a nonaminoglycoside antibiotic.

The enzymatic modification of aminoglycoside antibiotics results in the loss of antimicrobial activity; however, the mere presence of a modifying enzyme does not always confer resistance to aminoglycosides. This is because the rate of modification is also an important determinant of resistance (14, 15, 37). Examples of bacteria which can modify an aminoglycoside but nevertheless remain susceptible to the drug are known (5, 19, 37); resistance is not conferred by the modifying enzyme because the inactivation is inefficient. Thus, it is reasonable to expect that the inhibitors of aminoglycoside-modifying enzymes could be used as potentiators of aminoglycoside activity against strains harboring these enzymes (37).

We report here a natural product produced by Streptomyces neyagawaensis that potentiates the activity of certain aminoglycoside antibiotics against aminoglycoside-resistant bacteria possessing a 2"-O-adenylyltransferase. The compound has been identified as 7-hydroxytropolone (7-HT) and appears to be a specific inhibitor of the adenylyltransferase.

MATERIALS AND METHODS

Bacteria. Escherichia coli W771 strains and Klebsiella pneumoniae OK8 were kindly provided by Julian Davies, Biogen SA, Geneva, Switzerland. Staphylococcus aureus AP01 and S. aureus PALM were obtained from François Le Goffic, Centre National de la Recherche Scientifique, Thiais, France. All other strains were obtained from the Eli Lilly culture collection.

Preparation of 7-HT and derivatives. For most experiments reported here, 7-HT was prepared synthetically by persulfate oxidation of tropolone (Aldrich Chemical Co.) by the methods of Nozoe et al. (T. Nozoe, S. Seto, S. Ito, M. Sato, and T. Katono, Chem. Abstr. 49:8239b, 1955). 7-HT, 5-HT, and the dihydroxytropolones were separated from the reaction mixture by counter-current distribution with chloroform-toluene-methanol-water (15:15:23:7) (H. Kirst and G. Marcon, manuscript in preparation). The structure of 7-HT is given in Fig. 1.

An analogous persulfate oxidation of 5-dimethylallyltropolone was used to prepare 4-dimethylallyl-7-HT. 4-Isopropyl-7-HT was generously provided by G. Barton, Forintek Canada Corp., Vancouver, British Columbia. The remaining derivatives used (see Table 3) were prepared by published procedures: 7-bromotropolone (10), 7-nitrotropolone (11), 7-aminothropolone (Y. Kitahara, Chem. Abstr. 51:16398f, 1957), and 6-methyl-7-carboxytopolone (13).

The sample of 7-HT used for one experiment (see Fig. 5) was isolated from the fermentation broth of S. neyagawaensis by HP-20 and Sephadex G-10 chromatography (K. Michel and M. Anderson, unpublished methodology). This material had a purity of approximately 25%. Chemically pure compounds were used in all other experiments.

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Potentiation of aminoglycoside activity. The ability of 7-HT and derivatives to potentiate the antimicrobial activity of aminoglycoside antibiotics was determined by two methods: (i) by measuring changes in absorbance in T-soy broth as described in the legend to Fig. 2 or (ii) by measuring the zone of inhibition surrounding a sensitivity disk containing 7-HT which had been placed on T-soy agar supplemented with a subinhibitory concentration of aminoglycoside. In both cases, inhibition by the combination was compared with inhibition by each compound alone.

Preparation and assay of 2'-O-adenylyltransferase. E. coli W677/IR76.2 was grown to late-log phase, harvested, and osmotically shocked, using media and methods described by Haas and Dowding (20). Partially purified adenylyltransferase was prepared from the osmotically shocked cells by the methods of Goldman and Northrup (18). For most experiments, material desalted on a column of Bio-Gel P-2 after ammonium sulfate precipitation was used. In some cases, mentioned in the text, this material was further purified by chromatography on DEAE-agarose (DEAE-Bio-Gel A; Bio-Rad Laboratories) as described elsewhere (18).

Adenylyltransferase activity was assayed by the radiochemical assay (20). Reaction mixtures contained, in a final volume of 50 μl, TMND buffer (13 mM Tris, 8 mM magnesium chloride, 80 mM ammonium chloride, 2 mM dithiothreitol (pH adjusted to 8.0 with maleic acid), plus [3H]ATP, aminoglycoside, and enzyme protein at concentrations as indicated in the appropriate table and figure legends. Unless stated otherwise, reactions were incubated for 15 min at 30°C, and 25-μl samples were removed and applied to 25-cm Whatman P-81 phosphocellulose filter paper disks. Disks were washed in 70 to 80°C distilled water, rinsed, and dried. Protein concentrations were determined by the method of Lowry et al. (28), and radioactivity was measured with a liquid scintillation counter.

[^2]H]DHS accumulation. Bacteria were grown at 37°C in a low-osmolarity (22) tryptone-peptone broth (Difco Laboratories; tryptone, 3 g/liter; peptone, 5 g/liter; pH 7.2) to an optical density at 600 nm = 0.2. At this point, the culture was subdivided into 10-ml volumes. Preexposure to aminoglycosides was done by adding nonradioactive aminoglycosides with or without 7-HT to each flask and continuing the incubation for 20 min at 37°C in a shaking water bath. After preexposure, cells were collected by centrifugation and suspended in fresh tryptone-peptone broth. [3H]dihydrostreptomycin ([3H]DHS) (Amersham Corp.; 3.0 Ci/mmol) was added to a final concentration of 0.48 μCi/ml, and incubation was continued. At intervals, 0.5-ml samples were removed and pipetted onto 0.45-μm HAWP cellulose acetate and nitrate filters (Millipore Corp.) that had been presoaked in 0.1 M lithium chloride (22). Samples were washed three times with 0.1 M lithium chloride, dried, and counted in a scintillation counter.

![Figure 1](http://aac.asm.org/Downloaded_from/http://aac.asm.org)

**FIG. 1.** 7-Hydroxytropolone.

![Figure 2](http://aac.asm.org/Downloaded_from/http://aac.asm.org)

**FIG. 2.** Effect of aminoglycosides alone and in combination with 7-HT on growth of E. coli W677/IR76.2. T-soy broth was inoculated and incubated at 37°C with shaking until the optical density at 600 nm reached 0.13. The culture was subdivided into 10-ml volumes to which aminoglycosides and 7-HT were added as indicated. Absorbance was measured at 600 nm. Symbols: ▲, control cells (no addition); ■, aminoglycoside (10 μg/ml); □, 7-HT (5 μg/ml); ●, aminoglycoside (10 μg/ml) plus 7-HT (5 μg/ml). DKB, Dibekacin.
TABLE 1. Susceptibility of bacteria to aminoglycoside potentiation by 7-HT

<table>
<thead>
<tr>
<th>Strain</th>
<th>Enzyme activities present$^a$</th>
<th>Susceptibility to potentiation$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli W677/JR76.2</td>
<td>AAD(2'), APH(3'), APH(3&quot;)</td>
<td>+</td>
</tr>
<tr>
<td>K. pneumoniae OK8</td>
<td>AAD (2&quot;)</td>
<td>-</td>
</tr>
<tr>
<td>E. coli W677/R5</td>
<td>None</td>
<td>-</td>
</tr>
<tr>
<td>E. coli W677/JR225</td>
<td>AAC (6')</td>
<td>-</td>
</tr>
<tr>
<td>E. coli W677/R100</td>
<td>AAD (3&quot;)</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$ Abbreviations: AAD, aminoglycoside adenyltransferase; APH, aminoglycoside phosphotransferase; AAC, aminoglycoside acetyltransferase.

$^b$ The potentiation of tobramycin activity was determined by disk susceptibility as described in the text. Streptomycin was substituted for tobramycin to test for potentiation against E. coli W677/R100.

RESULTS

Potentiation of aminoglycoside activity by 7-HT. E. coli W677/JR76.2 possesses a 2"-O-adenylyltransferase (2"-O-nucleotidyltransferase) and at least two aminoglycoside phosphotransferases: a 3"-O-neomycin-kanamycin phosphotransferase and a 3"-O-streptomycin phosphotransferase (3, 18). The growth of this strain (Fig. 2) was unaffected by 10 μg of tobramycin, gentamicin, kanamycin A, dideoxykanamycin B (dibekacin), or butirosin per ml. However, a combination of either tobramycin, gentamicin, or dibekacin with 5 μg of 7-HT per ml was inhibitory. Since 7-HT alone was not inhibitory, and because tobramycin, gentamicin, and dibekacin are each substrates for the 2"-O-adenylyltransferase, the effectiveness of the combination can be explained as a potentiation of aminoglycoside activity by the inhibition of enzymatic modification.

The results in Fig. 2 indicate that some aminoglycosides were potentiated more readily than others. In particular, the combination containing tobramycin was more effective than a combination of 7-HT plus gentamicin or dibekacin. This could be a function of differences in enzyme affinity for the aminoglycosides.

Kanamycin A is a substrate for the adenyllyltransferase (3), yet the combination of 7-HT and kanamycin A did not inhibit growth. The most likely explanation for this is that kanamycin A is inactivated by the 3'-O-phosphotransferase in this strain, independent of the fact that 7-HT inhibits its adenylation. This, of course, requires that 7-HT does not inhibit the phosphotransferase reaction. In contrast to kanamycin A, dibekacin lacks the 3'-hydroxyl group which is phosphorylated by the 3'-O-phosphotransferase. Dibekacin could not be phosphorylated, and it was predictably potentiated by 7-HT. The fact that 7-HT failed to potentiate butirosin (a substrate for phosphorylation only) is further evidence that 7-HT does not affect the 3'-O-phosphotransferase.

In other experiments (data not shown), sisomicin and netilmicin were potentiated by 7-HT against E. coli W677/JR76.2. Both sisomicin and netilmicin are subject to 2"-adenylylation (29, 32). Thus, the aminoglycoside-potentiator activity of 7-HT appeared limited to those aminoglycosides that were substrates for the 2"-O-adenylyltransferase. Moreover, potentiation was demonstrated against other strains containing a 2"-O-adenylylating enzyme but not against aminoglycoside-susceptible strains or strains possessing other aminoglycoside-modifying enzymes (Table 1).

Enzyme inhibition. The limited spectrum of potentiation activity seen with 7-HT suggested that the mechanism of potentiation involved the inhibition of the 2"-O-adenylyltransferase. This enzyme was isolated from E. coli W677/JR76.2 by osmotic shocking as described above. As shown in Table 2, 7-HT inhibited the adenyllylation of tobramycin and other aminoglycoside substrates for this enzyme. Even though kanamycin A was not potentiated against E. coli W677/JR76.2 by 7-HT (Fig. 2), the formation of 2"-O-adenylylkamycin A was, nevertheless, inhibited by this compound. In a separate experiment (data not shown), the adenyllylation of sisomicin and netilmicin was likewise inhibited by 7-HT. 7-HT did not inhibit the 3'-O-phosphotransferase present in E. coli W677/JR76.2, nor was there any effect on the 3'-N-acetyltansferase isolated from E. coli W677/JR225 (J. Hobbs, Jr. and N. Allen, unpublished data).

TABLE 2. Inhibition of 2"-O-adenylyltransferase by 7-HT

<table>
<thead>
<tr>
<th>Substrate</th>
<th>7-HT concn (μg/ml)</th>
<th>pmol of substrate modified$^a$</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobramycin</td>
<td>0</td>
<td>235</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>116</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>66</td>
<td>72</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0</td>
<td>219</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>108</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>60</td>
<td>73</td>
</tr>
<tr>
<td>Kanamycin A</td>
<td>0</td>
<td>229</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>85</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>31</td>
<td>87</td>
</tr>
<tr>
<td>Dibekacin</td>
<td>0</td>
<td>221</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>174</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>90</td>
<td>60</td>
</tr>
</tbody>
</table>

$^a$ Assays were performed as described in the text, using 1 mM [14C]ATP (20 μCi/μmol), 1.25 μg of aminoglycoside, and 42 μg of enzyme protein per reaction mixture.

$^b$ —, None.
7-HT kinetics were performed as mixture. Estimated ATP was higher for different strains of E. coli. The assay procedure described in the text was modified by using 5 µg of enzyme protein per reaction mixture, tobramycin concentrations as indicated, and by reducing the reaction time to 0.5 min. The concentration of [14C]ATP (30 µCi/µmol) was 0.5 mM. These changes were made so that initial rates relative to maximum velocity could be measured at saturating ATP concentrations.
this position gave inactive derivatives. The 3,7- and 4,7-dihydroxy compounds were active as enzyme inhibitors, with the 4,7 derivative comparable to 7-HT. (The 4,7 derivative could not be separated completely from the 3,7 isomer.) Ring alkylation of 7-HT reduced inhibitory activity in some cases, e.g., 4-dimethylallyl-7-HT. Yet the activity of 4-isopropyl-7-HT was as good as, or better than, that of 7-HT. Thus, ring alkylation at the 4-position can yield an active enzyme inhibitor providing the substituent is not overly bulky. In all cases, derivatives that inhibited enzyme activity were also able to potentiate tobramycin activity against *E. coli* W677/76.2. Tropolone and other derivatives devoid of enzyme-inhibitor activity did not show such potentiation.

**Potentiation of aminoglycoside accumulation.**

Enzymatic modification in aminoglycoside-resistant bacteria is accompanied by a reduced rate of aminoglycoside uptake (7, 15, 23). If 7-HT potentiates the activity of tobramycin by inhibiting the 2'-O-adenyllyltransferase, we might expect 7-HT to facilitate an enhanced rate of tobramycin uptake in the enzyme-containing strain. We examined the effect of tobramycin and tobramycin plus 7-HT on aminoglycoside uptake (as measured by [3H]DHS uptake; 22) in a tobramycin-susceptible and an adenylylating strain. The results are given in Fig. 5.

Tobramycin-susceptible *E. coli* W677 (Fig. 5A) accumulated [3H]DHS after a lag of about 10 min. When these cells were preexposed to 1 μg of tobramycin per ml for 20 min, [3H]DHS uptake occurred at an enhanced rate with no lag. Uptake after preexposure to either 7-HT or to a combination of 7-HT plus tobramycin was not significantly different from control measurements.

The kinetics of [3H]DHS uptake in the adenylylating strain *E. coli* W677/JR76.2 were much different (Fig. 5B). Without preexposure to an aminoglycoside, very little [3H]DHS was accumulated during the 20-min measurement. This was expected since this strain possesses a streptomycin-2'-O-phosphotransferase (see below). Preexposure to 1 μg of tobramycin per ml enhanced the subsequent uptake of [3H]DHS, but the extent of this enhancement was only 5% of that seen in tobramycin-susceptible cells (Fig. 5A). However, preexposure to a combination of 7-HT plus tobramycin resulted in a much enhanced rate of [3H]DHS uptake. Since 7-HT alone had no effect, the enhanced rate of [3H]DHS uptake was likely due to the penetration of tobramycin resulting from the inhibition of the 2'-O-adenyllyltransferase.

The experiments in Fig. 5 were repeated with synthetically prepared 7-HT. The results were qualitatively identical to those shown.

**DISCUSSION**

Killing by aminoglycoside antibiotics has been described as a series of events that require a functional, energy-dependent transport process along with some form of drug interaction with the ribosome (7, 8, 21, 24). Aminoglycoside antibiotics are initially accumulated across the cytoplasmic membrane of susceptible bacteria by a low-affinity uptake system that is rate limiting for aminoglycoside susceptibility (8). Once across the cytoplasmic membrane, aminoglycoside antibiotics interact with a ribosomal binding site (1, 24). This latter event results in a dramatic increase in the rate of antibiotic uptake and quickly leads to the cessation of polypeptide synthesis and cell death (8). Both transport and ribosomal interactions of aminoglycoside antibiotics are altered in aminoglycoside-resistant bacteria possessing drug-modifying enzymes. Aminoglycoside modification by these bacteria is accompanied by reduced rates of drug accumulation (7, 15, 23). Presumably, all drug molecules that manage to penetrate and reach the ribosome are modified in transit (15, 23). Since the enhanced rates of aminoglycoside accumulation needed for killing seemingly require an interaction between the antibiotic and ribosome, and since the modified aminoglycoside is unable to interact with the ribosome (4), aminoglyco-

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**TABLE 3. Inhibition of 2'-O-Adenylyltransferase by tropolone and tropolone derivatives**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>% Inhibition of enzyme activity at μg/ml</th>
<th>100</th>
<th>10</th>
<th>0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tropolone</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7-Hydroxytropolone</td>
<td>100</td>
<td>78</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>5-Hydroxytropolone</td>
<td>59</td>
<td>&lt;0</td>
<td>&lt;0</td>
<td>9</td>
</tr>
<tr>
<td>3,7-Dihydroxytropolone</td>
<td>54</td>
<td>&lt;0</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>4,7-Dihydroxy/3,7-dihydroxytropolone (85:15)</td>
<td>94</td>
<td>50</td>
<td>&lt;0</td>
<td></td>
</tr>
<tr>
<td>4-Dimethylallyl-7-hydroxytropolone</td>
<td>65</td>
<td>21</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>4-Isopropyl-7-hydroxytropolone</td>
<td>100</td>
<td>76</td>
<td>31</td>
<td>—</td>
</tr>
<tr>
<td>7-Bromotropolone</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>7-Nitrotropolone</td>
<td>16</td>
<td>4</td>
<td>11</td>
<td>—</td>
</tr>
<tr>
<td>7-Aminotropolone</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>6-Methyl-7-carboxytropolone</td>
<td>0</td>
<td>9</td>
<td>12</td>
<td>—</td>
</tr>
</tbody>
</table>

*a* Assays were performed as described in the text with 0.4 mM [14C]ATP (10 μCi/μmol), 1.25 μg of tobramycin, and 42 μg of enzyme protein per reaction mixture.

*b* Tropolone:

*c* —, Not done.
FIG. 5. Effect of 7-HT on enhanced accumulation of [3H]DHS. (A) E. coli W677 or (B) E. coli W677/JR76.2 was preexposed to either tobramycin or a combination of tobramycin and 7-HT, after which the kinetics of [3H]DHS uptake were determined over a 20-min period as described in the text. Data represent total uptake. Symbols: 
- control cells (no preexposure); A, preexposure to tobramycin (1 μg/ml); B, preexposure to tobramycin (1 μg/ml) plus 7-HT (5 μg/ml). 7-HT used in this experiment was isolated from S. neyagawaensis (see the text).

Outside uptake remains depressed in these bacteria, and the cells survive. Obviously, the rate of drug modification is significant since resistance can be maintained only as long as this rate equals or exceeds the rate of drug uptake (15).

There are examples (5, 19, 37) of strains that are susceptible to certain aminoglycosides in spite of the fact that the strains possess an enzyme for modifying the drug. The explanation for susceptibility is based on the kinetic behavior of the enzyme. Although the aminoglycosides are subject to modification in these cases, they are poor substrates. The $K_m$ for modification is sufficiently high so that unmodified antibiotic can reach the ribosome. As a result of observations such as these, it has been predicted (37) that an enzyme inhibitor able to increase the $K_m$ for an aminoglycoside could confer susceptibility to that drug in an enzyme-containing resistant strain.

Aminoglycosides and compounds structurally related to aminoglycosides have been reported to inhibit aminoglycoside-modifying enzymes (2, 16, 35, 36). In at least one case, an amino sugar (3-amino-3-deoxy-D-glucose) that inhibited the phosphorylation of kanamycin $A$ was able to increase the antibacterial activity of kanamycin $A$ against phosphorylating strains (35). Analogs of ATP, including formycin $A$ and B, also were reported to inhibit the same phosphotransferase (16). More recently, Williams and Northrup (38) described a synthetic, multisubstrate analog inhibitor of a gentamicin acetyltransferase. Although this compound was a potent enzyme inhibitor, it did not potentiate gentamicin activity against resistant bacteria.

In this report, we have described 7-HT, a non-aminoglycoside produced by S. neyagawaensis that inhibits aminoglycoside-2'-O-adenyltransferase. Although 7-HT has broad-spectrum antibiotic activity (M. Hoehn and J. Ott, unpublished data; 25), its activity against aminoglycoside-resistant E. coli is poor. However, when combined with aminoglycosides subject to adenylation, resistant strains possessing the 2'-O-adenyltransferase were inhibited. This enzyme is commonly encountered as a mechanism of gentamicin inactivation (33) and reportedly accounts for much of the gentamicin-tobramycin resistance found in hospital-acquired Klebsiella infections (39). Its widespread nature may be accounted for, in part, by the fact that the enzyme determinant has been found associated with a transposon in Klebsiella sp. (31).

Enzyme inhibition and potentiation by 7-HT seems to be limited to those strains that contain an adenylyltransferase. It is interesting that 7-HT did not inhibit the 3'-O-phosphotransferase present in E. coli W677/JR76.2 even though ATP is a cosubstrate for this enzyme. In preliminary experiments (unpublished data), 7-HT potentiated tobramycin against S. aureus AP01 (26), possessing a 4'-O-adenyltransferase. Although 7-HT has not been tested directly for the inhibition of this enzyme, these results raise the possibility that the inhibitory activity of 7-HT may not be limited to 2'-O-adenylation. However, 7-HT did not potentiate aminoglycoside activity against S. aureus PALM (27), which possesses a 2'-O-phosphotransferase.

In the past, the usual approach to the problem of aminoglycoside resistance was to chemically modify aminoglycoside structures to generate nonsubstrate derivatives. This approach led to the development of amikacin (the 1-N-aminohydroxybutyl derivative of kanamycin A) and netilmicin (the 1-N-ethyl derivative of sisomicin). Both semisynthetic drugs resist inactivation by several aminoglycoside-modifying enzymes (29, 32). The inhibition of the modifying enzyme to potentiate aminoglycoside antibiotics against resistant bacteria is an alternative solution to this problem. The activity of 7-HT is thus similar to the activity of clavulonic acid (30) or the penicillanic acid sulfone (sulbactam; 17) which are inhibitors of $\beta$-lactamases. 7-HT could be a prototype of low-molecular-weight inhibitors of aminoglycoside-inactivating enzymes.
7-HT inhibits adenyllylation by competing with ATP for the enzyme. An inhibitor that was fully competitive with the aminoglycoside, rather than ATP, might be a better potentiatior since the apparent K_m for the aminoglycoside should be increased. Nevertheless, a combination of 7-HT plus tobramycin was noticeably more effective than either compound alone in treating mice infected with *K. pneumoniae* OKR8 possessing the 2'-O-adenylyltransferase (F. Counter, unpublished data).

The finding that the inhibition of the 2'-O-adenylyltransferase facilitates the penetration of tobramycin (Fig. 5) reemphasizes both the significance of the role of the modifying enzyme in aminoglycoside resistance and the importance of a ribosomal drug interaction in producing enhanced uptake. The effectiveness of the combination of 7-HT plus tobramycin is due to the binding of unmodified tobramycin at the ribosome which triggers the enhanced rates of uptake needed for killing. In the absence of 7-HT, only adenyllytobramycin should reach the ribosome. The enhanced rate of [3H]DHS uptake must result from tobramycin penetration, since 7-HT alone has no effect and [3H]DHS cannot facilitate its own uptake since it is phosphorylated by *E. coli* W6771JR76.2. Although aminoglycoside uptake is very poor in bacteria that modify these drugs, it is clear from the work here that enzymatic modification is the key determinant of resistance.

ACKNOWLEDGMENTS

We thank M. Anderson, J. Martin, G. Marconi, K. Michel, H. Percifield, and J. Toth for their contributions to this study.

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