Electrostatic Interactions in RNA Aminoglycosides Binding

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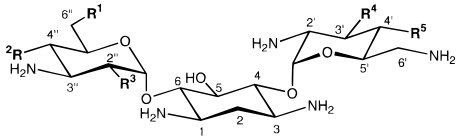
Aminoglycoside antibiotics interact with seemingly unrelated families of RNA molecules. They bind to 16S ribosomal RNA and interfere with the decoding mechanism by distorting the codon−anticodon recognition.[1,2] Similar aminoglycosides specifically inhibit self-splicing group I introns[3] and the hammerhead ribozyme.[4] Recently, these antibiotics have been found to competitively block the binding of the HIV Rev protein to its viral RNA recognition site (RRE), thereby inhibiting virus production.[5] While the molecular details of these important RNA−drug interactions are yet to be elucidated,[6] aminoglycosides provide an entry into the largely unexplored area of RNA−small-molecules recognition.[7]

As highly functionalized polycationic oligosaccharides, interactions between polar residues of the aminoglycosides (*i.e.*, amino and hydroxyl groups) and the RNA backbone and/or heterocyclic bases are likely to occur.[8] The reported structure−activity relationships for the natural aminoglycosides suggest that electrostatic interactions are important for RNA binding.[3−5] The most active derivatives contain at least 5 or 6 amino groups that are predominantly charged at pH 7.0.[9] The role played by the hydroxyl groups is much less clear. Kanamycin B is 20-fold less active than tobramycin (its 3′-deoxy derivative) in inhibiting self-splicing of group I introns,[3] and a similar trend has been found in the inhibition of Rev−RRE binding.[5]

Will the removal of additional hydroxyl groups enhance RNA binding? Comparing the basicity of ethylamine ($pK_a = 10.7$) to ethanolamine ($pK_a = 9.50$)[10] indicates that the presence of a vicinal hydroxyl lowers the basicity of the amine by more than one pK_a unit. We therefore hypothesized that deoxygenated aminoglycoside antibiotics may be stronger RNA binders due to an increased basicity of the neighboring amino groups. The aminoglycosides studied are shown in Figure 1 and include the following: kanamycin B (**1**), tobramycin (**2**), dibekacin (4′-deoxytobramycin, **3**), 6″-deoxytobramycin (**4**), 4″-deoxytobramycin (**5**), and 2″-deoxytobramycin (**6**). This series represents a complete set of aminoglycoside derivatives in which the hydroxyl groups are removed one at a time while the remaining functional groups are kept intact.[11]



	Aminoglycoside	R^1	R^2	R^3	R^4	R^5
1	Kanamycin B	OH	OH	OH	OH	OH
2	Tobramycin	OH	OH	OH	**H**	OH
3	Dibekacin (4′-Deoxytobramycin)	OH	OH	OH	**H**	**H**
4	6″-Deoxytobramycin	**H**	OH	OH	**H**	OH
5	4″-Deoxytobramycin	OH	**H**	OH	**H**	OH
6	2″-Deoxytobramycin	OH	OH	**H**	**H**	OH
7	6″-Amino-6″-deoxytobramycin	**NH_2**	OH	OH	**H**	OH

Figure 1. Natural and synthetic aminoglycosides studied.

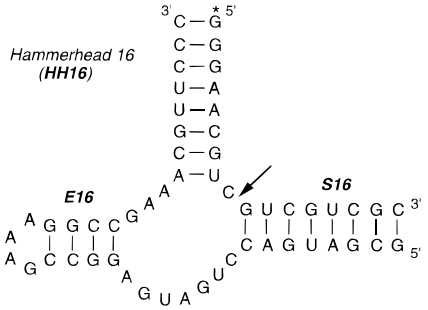


Figure 2. Secondary structure of the hammerhead ribozyme (HH16),[4,12] showing the enzyme E16 and the ^{32}P 5′-labeled substrate S16. The arrow indicates the cleavage site.

The natural and modified aminoglycosides have been tested for their ability to inhibit the hammerhead ribozyme (Figure 2).[12,13] At pH 7.3, the ribozyme E16 cleaves its substrate S16 with a pseudo-first-order rate constant of 0.075 min^{-1} (Table 1 and Figure 3).[11] At a 100 μM aminoglycoside concentration, tobramycin (**2**) decreases the cleavage rate 6-fold. Dibekacin (**3**) and 4″-deoxytobramycin (**5**), lacking a single secondary hydroxyl each, decrease the cleavage rate by a factor of 20. 2″-Deoxytobramycin (**6**), lacking the other secondary hydroxyl on the 3″-amino-3″-deoxy-α-D-glucopyranosyl ring is found to be the most potent inhibitor among the deoxy derivatives, resulting in an almost 40-fold decrease in the cleavage rate. These deoxygenated derivatives are therefore far better inhibitors of the hammerhead ribozyme when compared to tobramycin, the parent aminoglycoside, and reach the level of neomycin B (**8**), the strongest inhibitor hitherto known.[4] In contrast, 6″-deoxytobramycin (**4**), lacking the only primary hydroxyl in the tobramycin skeleton, is less effective than tobramycin (**2**), slowing down the ribozyme only 4-fold, similar to kanamycin B (**1**), the least active aminoglycoside tested.[14]

The most potent ribozyme inhibitors are the deoxygenated aminoglycoside derivatives lacking the secondary hydroxyl groups (Table 1). The 4′-OH is part of a 3-aminopropanol fragment and is likely to interact with the primary 6′-amino group. The 4″- and 2″-hydroxyls are vicinal to the 3″-amino group. Additionally, the 2″-OH is within a hydrogen-bonding

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(14) Lowering the concentration of the aminoglycosides to 10 μM results in a similar trend (Table 1).

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Table 1. Pseudo-First-Order Rate Constants for the Cleavage Step (k_2) of the Hammerhead Ribozyme in the Presence of Aminoglycoside Antibiotics **1**−**8**[a]

aminoglycoside derivative	k_2 (min^{-1}) pH 7.3		k_2 (min^{-1}) pH 6.8	
	100 μM	10 μM	100 μM	10 μM
none	0.075		0.045	
1	0.018	0.060	0.008	0.028
2	0.012	0.051	0.004	0.026
3	0.004	0.046	0.003	0.035
4	0.017	0.052	0.006	0.035
5	0.004	0.039	0.004	0.032
6	0.002	0.034	0.003	0.028
7	*b*	0.011	*b*	0.004
8[c]	*b*	0.018	*d*	*d*

[a] Initial rates were measured under single turnover conditions in the presence of subsaturating Mg^{2+} concentration and subsaturating ribozyme.[11] The values given are the average of three independent experiments, and the error is estimated to be 10%. [b] Complete inhibition is observed. [c] Neomycin B. [d] Not determined.

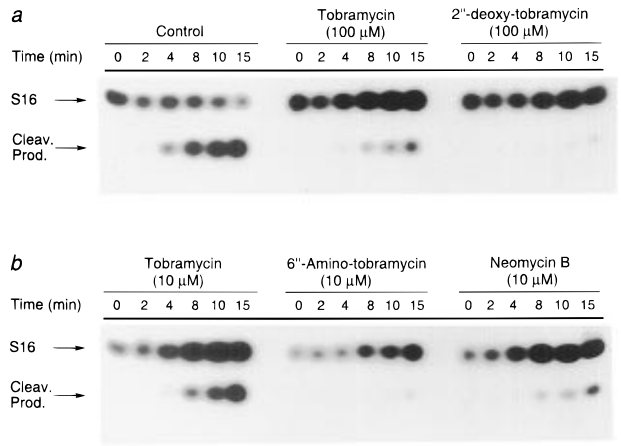

Figure 3. An autoradiogram of a 20% polyacrylamide gel used to separate the products of the time-dependent hammerhead ribozyme cleavage reactions at pH 7.3.[11]

distance from the 1-amino group on the 2-deoxystreptamine ring.[15] Therefore, removal of the 4′- or the 4″-hydroxyl may enhance the basicity of the corresponding neighboring amine group, while deoxygenation of the 2″-hydroxyl may affect two amino groups simultaneously, supporting the superior inhibitory activity observed for **6**. The 6″-hydroxyl is remote to any amino group, and its removal does not enhance RNA binding. While several factors may contribute to the trends observed, we suggest that altering the basicity of the amino groups on the antibiotics is the important mechanism for tuning their affinity to RNA. In fact, the higher activity of tobramycin (**2**) compared to that of kanamycin B (**1**) in inhibiting the self-splicing of group I introns[3] and Rev−RRE binding[5] can be attributed to similar effects: tobramycin lacks the equatorial 3′-hydroxyl present in kanamycin B and is likely to display a higher charge density toward the RNA binding site.

If the degree of protonation is the dominant factor distinguishing kanamycin B from tobramycin, and tobramycin from its deoxy derivatives, lowering the pH should compensate for these differences. Indeed, repeating the experiments described above at pH 6.8 rather than 7.3 clearly showed that the differences in inhibitory activity of the various derivatives have been narrowed significantly (Table 1). These observations support our hypothesis that altering the pK_a of the amino groups is the likely mechanism for modulating the RNA affinity of the various deoxy aminoglycosides studied.

To further test our hypothesis, we have synthesized 6″-amino-6″-deoxytobramycin (**7**), where the primary hydroxyl was substituted with a basic primary amine (Figure 1). At 100 μM and pH 7.3, **7** completely inhibits the hammerhead ribozyme and no substrate cleavage is observed. At 10 μM concentration, where tobramycin (**2**) and its deoxy derivatives (**3**−**6**) show very little inhibitory activity, 6″-amino-6″-deoxytobramycin (**7**) decreases the ribozyme cleavage rate 7-fold (Figure 3b, Table 1). Under the same conditions, neomycin B slows down the ribozyme cleavage rate only 4-fold (Figure 3b and Table 1). The novel 6″-amino-6″-deoxytobramycin (**7**) is therefore the first synthetic aminoglycoside analog to show higher inhibitory activity than neomycin B, the best RNA binder previously reported.

In summary, our data provide strong experimental support for the critical role of electrostatic interactions in RNA aminoglycoside binding. Revealing the interplay between hydroxyl and their neighboring ammonium groups not only clarifies previously unexplained trends among the naturally occurring aminoglycosides but also provides leads for the design of novel RNA binders with superior activity. These low-molecular-weight molecules that target pivotal RNA sites are favorable candidates for drug discovery.

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Supporting Information Available: Synthetic schemes and analytical data for all new derivatives as well as complete experimental procedures and data for evaluating ribozyme kinetics and RNA binding (12 pages). See any current masthead page for ordering and Internet access information.

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