Deoxystreptamine Dimers Bind to RNA Hairpin Loops

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The functions of RNA in the cell are vast and varied. The original discovery of mRNA accorded RNA the important if somewhat pedestrian role as the intermediary between DNA and protein. However, over the ensuing decades it has become apparent that RNA is more than a transient repository of genetic information but instead has pivotal roles in cell division, gene expression, and catalysis. Indeed, regulation of a variety of processes with small untranslated RNAs has emerged as a general theme in biology.1 However, over the ensuing decades it has become apparent that

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Accordingly, there has been considerable interest in the identification of small molecules that bind tightly to RNA.3 RNA-binding natural products such as aminoglycosides and macrolides are widely used as antibiotics, and RNA-binding small molecules have potential against a variety of biological targets.4 However, in contrast to DNA,5 no general paradigm currently exists by which sequence-specific RNA-binding small molecules can be designed. Efforts toward this goal are complicated by the variety of secondary structures that RNA can adopt and by the fact that compounds specifically recognizing these RNA secondary structures are lacking. One successful approach has been the use of aminoglycosides to bind RNA from a variety of sources. In general, aminoglycosides recognize secondary structural elements within RNA in which the usual base pairing has been disrupted such as bulges, internal loops, and stem junctions.6 For instance, the aminoglycoside neomycin will bind tightly to bulge regions of unrelated RNA sequences from the 16S ribosome, HIV TAR, HIV RRE, and the Group I intron in the low micromolar range.7 However, despite their promiscuity for these types of RNA secondary structures, aminoglycosides do not typically bind to RNA hairpin loops,6c which are a major RNA secondary structural motif.8 Indeed, there are no general RNA hairpin loop-binding compounds. Herein we report the results of a systematic study designed to identify general RNA hairpin loop-binding small molecules.

Deoxystreptamine (1) has been reported to bind weakly to two base units within a disrupted RNA helix.9 Solution studies carried out with 1 have shown that it will bind to 5′-3′ two-base steps (including GU, UG, and GG), albeit with a very low affinity (>1 mM).3 We reasoned that linkage of two such units by an appropriate tether could lead to compounds with the requisite flexibility and functionality to bind RNA hairpin loops. Thus, we have tethered together two molecules of 1 with various linkers to create deoxystreptamine dimers, and we show that these molecules have the capacity to bind to a variety of RNA hairpin loops that are not recognized by aminoglycosides.

To construct the desired dimers, the enantiopure diacetate diazide of deoxystreptamine was created through known protocols (2 in Scheme 1).10 Protecting group manipulation provided alcohol 3, the appropriately protected precursor for dimerization. Treatment of 3 with sodium hydride, followed by addition of a variety of dibromides gave the protected dimers 4–14. Deprotection of the acetonide with AcOH provided tetraazides 4a–14a, and azide reduction with PMe3/NaOH furnished the desired deoxystreptamine dimers 4b–14b (Scheme 1). To assess the ability of these deoxystreptamine dimers to bind hairpin RNA, we utilized RNAs with tetra-, hexa-, hepta-, and octaloops.11 All these RNAs had 5′G-U3′ steps on either side of the loop and a common five base pair stem consisting of alternating G–C and C–G bases (Table 1). To detect binding, all of the RNA sequences were synthesized with a 3′-fluorescein label such that binding could be monitored by a change in the fluorescence signal.12

The results from these binding experiments are displayed in Table 1, and a representative graph comparing a dimer of 1 and an aminoglycoside is displayed in Figure 1. As expected, deoxystreptamine itself and all aminoglycosides tested had very high dissociation constants (Kd > 1 mM).13 However, several of the deoxystreptamine dimers had significant affinities for the RNA hairpin loops. For dimers containing both the aromatic and aliphatic linkers, binding was favorable for longer tether lengths. In addition, compounds containing the aromatic linkers generally showed tighter binding over their aliphatic counterparts.

Several controls established the importance of the dimeric nature of deoxystreptamine for RNA binding. Monomer 1 showed no detectable binding. In addition, while compound 11b bound the RNAs, the tetraazide analogue 11a showed no binding. Finally, neither the linkers alone, nor a linker with a single monomer attached (compound 15) showed significant binding to the RNA hairpin loops. Taken together, the data indicate that dimeric versions of deoxystreptamine are necessary for binding. In addition, the experiments with the tetraazide 11a suggests that the binding observed is due to direct interactions between the amines of deoxystreptamine and the RNAs.

To assess if these newly identified RNA–small molecule interactions were general for loops of different sequences, nine variants of RNA heptaloop C were created, and the binding of these RNAs to 14b was determined. All of these loops essentially have the same affinity for 14b (~6 μM, see Supporting Information for details); thus, the binding appears to be independent of the primary loop sequence. In addition, a competition experiment was performed in which binding of 14b to two of the hairpin loops was assessed in the presence of a large excess of the stem. In both cases, no change in Kd was observed, indicating that the deoxystreptamine dimer is not binding to the stem.

This 6 μM binding affinity of 14b with loop C rivals that of standard aminoglycoside–RNA pairs.6c To establish the precise location of this small molecule–nucleic acid interaction, an RNA
footprinting experiment was conducted. Loop C was 3′ radiolabeled with γ-32P-ATP and incubated with various concentrations of 14b. The gel in Figure 2 clearly reveals a strong protection from RNase digestion in the loop region. Analysis of the G10 band of this gel potentially be paired with binders of other RNA secondary structural elements to obtain even higher affinities.14

In summary, we have shown that simple dimers of deoxystreptamine show strong binding to RNA hairpin loops, a secondary structural element that is not generally recognized by standard RNA-binding molecules such as aminoglycosides. Further explorations into the relationship between loop size and linker type, as well as applications of these molecules to RNAs of biological interest, are ongoing and will be reported in due course.

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Supporting Information Available: Full experimental protocols and characterization data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(11) Stable RNA stem-loops were designed using mfold (Mathews, D. H.; Sabrina, J.; Zuker, M.; Turner, D. H. J. Mol. Biol. 1999, 288, 911). The corresponding pentaloop showed multiple structures in mfold and was thus not utilized in this study.
(13) Absence of aminoglycoside binding was also confirmed by RNase I footprinting.
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