RNA-Selective Coordination Complexes Identified via Dynamic Combinatorial Chemistry

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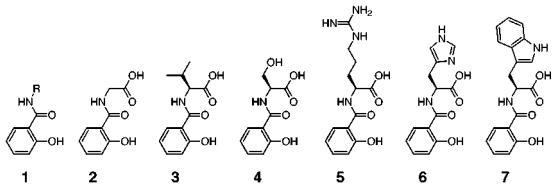
Small molecules that bind RNA are of exceptional importance: as fundamental guides in understanding molecular recognition, as tools for sequence- and tertiary structure-selective modification and mapping,[1] and as potential new therapeutic agents.[2] The majority of RNA-binding small molecules identified to date are aminoglycoside natural products,[3] or compounds based on the aminoglycosides.[4] While efforts by a number of groups to identify novel RNA-binding compounds have produced several notable successes, selectivity for RNA over binding to homologous DNA sequences has rarely been demonstrated.[5] As part of a general program aimed at the development of new methods for the design, synthesis, and identification of new compounds targeting a range of biologically relevant molecules, our group recently reported the identification of DNA-binding zinc salicylaldimine complexes from dynamic combinatorial libraries.[6] Dynamic combinatorial chemistry employs a mixture of compounds formed under conditions of thermodynamic equilibrium.[7] Placing such a dynamic combinatorial library (DCL) in solution with a target receptor causes this equilibrium to shift based on the binding energies of the individual library members, as those that bind the tightest are removed from the main pool of compounds in solution. In essence, this is a form of templated synthesis,[8] in which the receptor templates the synthesis of its own ligand. We now report the extension of this methodology to RNA binding.

Salicylamides (**1**) were selected as the ligands for construction of our RNA-binding library, since these would provide both metal-binding functionality and a variable position ("R") for incorporation of potential RNA-binding moieties. Since regulation of RNAs by proteins is well-known, the use of amino acids as "R" groups seemed a natural choice.[9] Compounds **2**−**7** were synthesized by standard procedures. We considered several metal ions as potential



elements around which individual components of the DCL could organize. Our requirements were that the metal not react with RNA at typical DCL selection concentrations and that it be capable of forming spectroscopically detectable, structurally well-defined complexes with a variety of salicylamides. $Cu^{(2+)}$ seemed an ideal choice, since several examples of mono- and bis-(salicylamide) copper complexes are known in the literature,[10] and cleavage of oligonucleotides by $Cu^{(2+)}$ complexes typically requires high temperatures or an added reductant and molecular oxygen.[11] We have examined the affinities of **4**, **6**, and **7** for Cu^{2+}-by UV titration and find these to be in the range of 0.2−0.4 mM. Thus, metal affinity is not strongly influenced by side chain identity. Assuming the formation of square-planar mono- and bis(salicylamide) complexes, a dynamic library formed from **2**−**7** would be expected to consist of at least 27 constitutionally distinct metal complexes. Of course, since other modes of coordination are also possible, it is likely that the actual library size is much larger.

We synthesized an RNA hairpin with the sequence 5′-UAGUCUUUCGAGACUA-3′ by standard automated methods. This sequence is derived from the GTP-binding P7 helix from the *Pneumocystis carinii* Group I intron,[12] with the sequence -UUCG- inserted to encourage duplex stability through hairpin formation (Figure 1). For comparison, the homologous DNA sequence (5′-TAGTCTTTCGAGACTA-3′) was also synthesized.

We employed an equilibrium dialysis protocol for library selection experiments. A Slide-a-Lyser dialysis tube (Pierce, Inc.; 3500 MWCO) was filled with 100 μL of a 100 μM solution of the receptor (DNA or RNA) in H15-Mg buffer,[13] or a blank buffer solution as control. This dialysis tube was immersed in a 300 μL solution of **2**−**7**, each at a concentration of 500 μM, in H15-Mg buffer. Each experiment was conducted in duplicate. To compare the effect of complexation on the observed selection, dialyses were carried out in the presence and absence of 3 mM $CuCl_2$. Each dialysis experiment was allowed to proceed for 12 h. The dialysis tube containing the receptor (or blank control) was then removed and placed in 400 μL of buffer for 12 h to dialyze away bound compounds. The dialysis tube with receptor solution was then reintroduced to a freshly prepared library solution (again, with or without $Cu^{(2+)}$) and the process repeated. After carrying out the dialysis versus library and subsequent dialysis versus buffer procedure a total of three times, the dialyzed ligand solutions were combined, lyophilized, and analyzed by reverse-phase HPLC.

Results of these experiments are shown in Figure 2. To determine which ligands were selected by binding to the receptor, ligand areas as determined by HPLC were normalized to those

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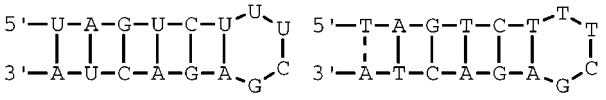

Figure 1. RNA and DNA hairpin sequences.

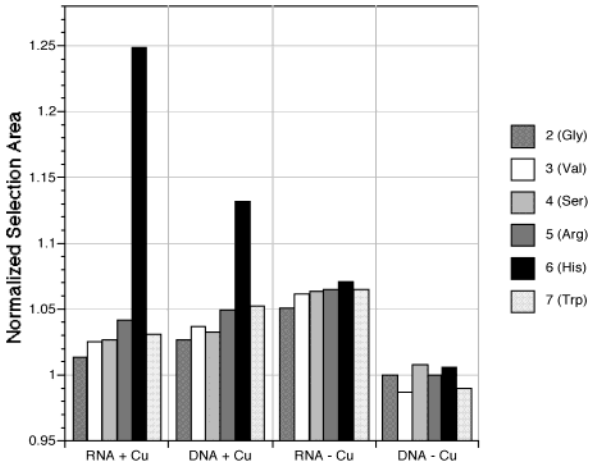

Figure 2. Results of Selection Experiments.

Table 1. Measured Affinities to RNA and DNA Hairpins (pH 7.5)

	K_D (nM)	
	RNA	DNA
2	ND	ND
2 + Cu	793	ND
5	> 5,000	> 6,000
5 + Cu	493	> 6,000
6	> 5,000	ND
6 + Cu	152	> 20,000

observed for dialyses carried out in the absence of receptor. As expected, none of the compounds **2**−**7** bind to either the DNA or RNA hairpins in the absence of $Cu^{(2+)}$, as evidenced by normalized peak areas which are at or near 1.0 (i.e., no difference in dialyzed ligand concentrations is observed in the presence or absence of receptor). However, dialyses conducted in the presence of $Cu^{(2+)}$ show a significant increase in the amount of **6** selected, as well as an intriguing and experimentally significant difference in the amount of **6** selected by the RNA sequence versus the amount selected by the DNA sequence. The fact that **6** emerges as the ligand most strongly selected in the presence of $Cu^{(2+)}$ rather than the positively charged **5** is intriguing, and suggests that selection is due to a specific recognition process.

To independently verify the ability of **6** to bind to the RNA hairpin in the presence of $Cu^{(2+)}$, as well as determine its selectivity for RNA relative to DNA, we measured its affinity by UV titration. As shown in Table 1 and Figure 3, **6** binds the RNA hairpin with remarkable affinity (152 nM) in the presence of $Cu^{(2+)}$, and moreover with *greater than 300-fold selectivity over the homologous DNA sequence*. Our measurements also indicate that **6** binds to the RNA sequence in the presence of $Cu^{(2+)}$ 3.2 times as tightly as does **5** + $Cu^{(2+)}$, and 5.2 times as tightly as does **2** + $Cu^{(2+)}$.

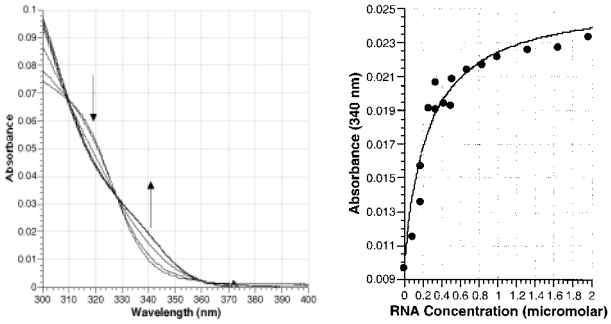

Figure 3. UV titration data for **6** binding to the RNA hairpin in the presence of 25 μM $Cu^{(2+)}$, and saturation profile at 340 nm.

The observation that **6** + $Cu^{(2+)}$ is selected by DNA in the dynamic diversity experiment (albeit much less strongly than by RNA) but does not appear to bind in the UV titration is somewhat surprising. One possibility is that **6** + $Cu^{(2+)}$ binds to the DNA hairpin nonspecifically or, alternatively, binds in a fashion that is not detectable by UV.

Job plot analysis of the complexation of $Cu^{(2+)}$ by **6** indicates that the stoichiometry of the primary species in solution is 1:1. In the only published example of an X-ray crystallographic structure for a $Cu^{(2+)}$−nucleoside complex, copper coordinates to guanosine N7 and a phosphate oxygen of guanosine-5′-monophosphate.[14] If this was the binding mode for the (**6**)−$Cu^{(2+)}$ complex, we would then expect to observe similar affinities for (**6**)−$Cu^{(2+)}$ to the DNA and RNA hairpins. Assuming that **6** occupies at most three coordination sites on $Cu^{(2+)}$, this suggests that the strong selectivity for RNA binding is perhaps due to direct coordination of the ribosyl 2′-OH to the metal. Of course, differences in conformation of the RNA and DNA hairpins (i.e., A-form vs B-form) and groove shape may also be important. Importantly, RNA binding by the (**6**)−Cu^{2+} complex in MES buffer at pH 6.0 (at which point the histidine imidazole is expected to be protonated and not available for Cu^{2+} coordination) was found to occur with a K_D of 50 nM.

In summary, we have employed dynamic combinatorial chemistry to select a compound which binds an RNA hairpin with high affinity and with extraordinary selectivity relative to a homologous DNA hairpin. These experiments suggest that DCLs will be generally useful for the identification of novel RNA-binding compounds.

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Supporting Information Available: UV titration experiments (raw data and Scatchard analyses), conditions for HPLC analysis of libraries, and concentration-independent melting profiles for DNA and RNA hairpins (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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