J|A|C|S
COMMUNICATIONS

Published on Web 11/26/2003

Application of NMR SHAPES Screening to an RNA Target

Eric C. Johnson,[†] Victoria A. Feher,[†,§] Jeffrey W. Peng,[‡] Jonathan M. Moore,*[,‡] and James R. Williamson*[,†]

Departments of Chemistry and Molecular Biology and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, and Vertex Pharmaceuticals, Inc., 130 Waverly Street, Cambridge, Massachusetts 02139

Received July 23, 2003; E-mail: jonathan_moore@vrtx.com; jrwill@scripps.edu

NMR spectroscopy has become a powerful tool to complement more traditional drug discovery efforts. Numerous NMR techniques have been developed in the past several years to aid in the identification of lead compounds that bind to target macromolecules.[1] Most of these efforts have focused on finding small molecules that bind to and inhibit the actions of protein enzymes or proteins involved in signaling pathways. Only recently have drug discovery efforts been applied to target RNA molecules, based on the increase in available structural and biochemical data on the many complex roles of RNA, suggesting the viability of RNA as a drug target.[2] In this study, we have applied some of the NMR screening methods more commonly used for protein targets to determine how effective they are for RNA targets.

The SHAPES screening library used for this study consists of 112 compounds that contain molecular scaffolds and side chains common to drug molecules.[3] Of these 112 SHAPES compounds, 44 are positively charged and 24 are negatively charged. The SHAPES library provides a diverse set of molecular shapes that can be useful in probing the dimensions of the target binding pocket. This library has been successfully used to find lead series for several protein targets.[4] This work represents the first application of SHAPES screening for an RNA target.

The RNA target we used is the P4P6 domain of the *Tetrahymena thermophila* Group I intron. This RNA consists of 160 nucleotides and has a well-defined, stable globular fold that contains several pockets where a small molecule ligand could potentially bind, as demonstrated by its 2.8 Å crystal structure.[5]

We investigated three different NMR screening methodologies: the saturation transfer difference (STD),[6] the 2D trNOESY,[7] and the WaterLOGSY[8] experiments. The 2D trNOESY and the WaterLOGSY methods both monitor the free ligand resonances and detect changes in the sign of the NOE transfer of magnetization for a rapidly tumbling free ligand versus a slowly tumbling ligand interacting with a macromolecule. In both experiments, negative resonance peaks are expected for freely tumbling ligands, whereas ligands interacting with a macromolecule will give rise to positive peaks (or negative peaks of reduced intensity because of the presence of both free and bound ligands).

Figure 1 shows representative WaterLOGSY and 2D trNOESY spectra of one of the SHAPES mixtures containing four potential ligands. The inversion of the WaterLOGSY signal for compound 53 in the presence of P4P6 clearly indicates that it is interacting with the RNA. Binding of this compound with P4P6 is also demonstrated by the positive NOE cross-peaks between the resonances at 6.9 and 7.6 ppm in the 2D trNOESY spectrum. In general, we found that the analysis of the WaterLOGSY data was much clearer than that of the 2D trNOESY data. In most cases, the 2D trNOESY cross-peaks were only marginally visible above the baseline noise. Additionally, any cross-peaks near the diagonal are usually obscured by the much greater intensity of the diagonal peaks.

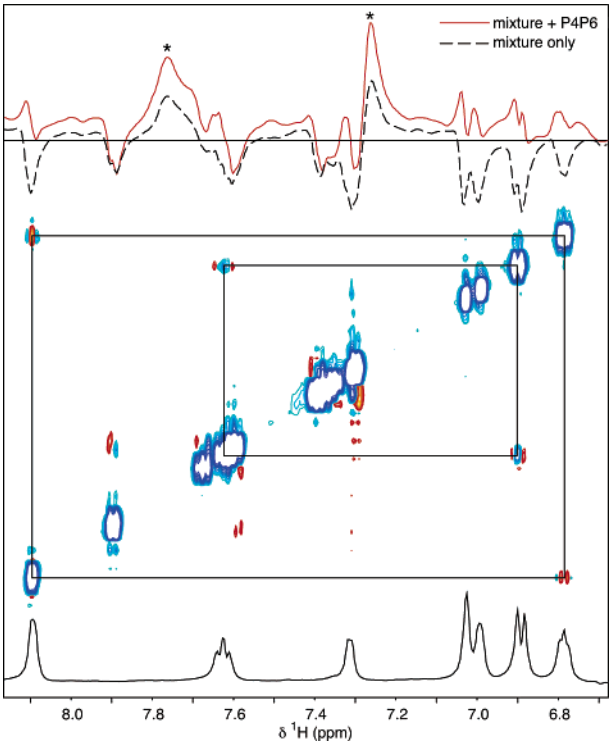

Figure 1. NMR screening spectra of mixture 14. WaterLOGSY spectra are shown at the top. The ligand mixture alone spectrum is shown in black, and the mixture in the presence of P4P6 RNA is shown in red. In the middle is the 2D trNOESY spectrum of the same mixture with P4P6. Positive contours are drawn in blue, and negative contours are drawn in red. NOE cross-peaks for the hit "compound 53" are indicated. The 1D reference spectrum for compound 53 is shown below. Each of the above samples contained 1 mM ligand, and those samples with RNA contained 50 μM P4P6. The positive peaks marked with asterisks (∗) in the WaterLOGSY are due to exchanging protons and are therefore not visible in the 2D trNOESY spectrum which was recorded in D_2O.

We also attempted to apply the STD method, in which a single resonance frequency on the macromolecule is directly irradiated. Magnetization is transferred throughout the macromolecule by spin diffusion and can then be transferred to a bound ligand through an intermolecular NOE. When screening for protein ligands, the STD experiment is often extremely sensitive and can be performed with much smaller quantities of protein than other NMR screening techniques.[1] Although the STD experiment has been demonstrated

[†] The Scripps Research Institute.
[‡] Vertex Pharmaceuticals.
[§] Present address: Quorex Pharmaceuticals, 1890 Rutherford Road, Suite 200, Carlsbad, CA 92008.

10.1021/ja037499s CCC: $25.00

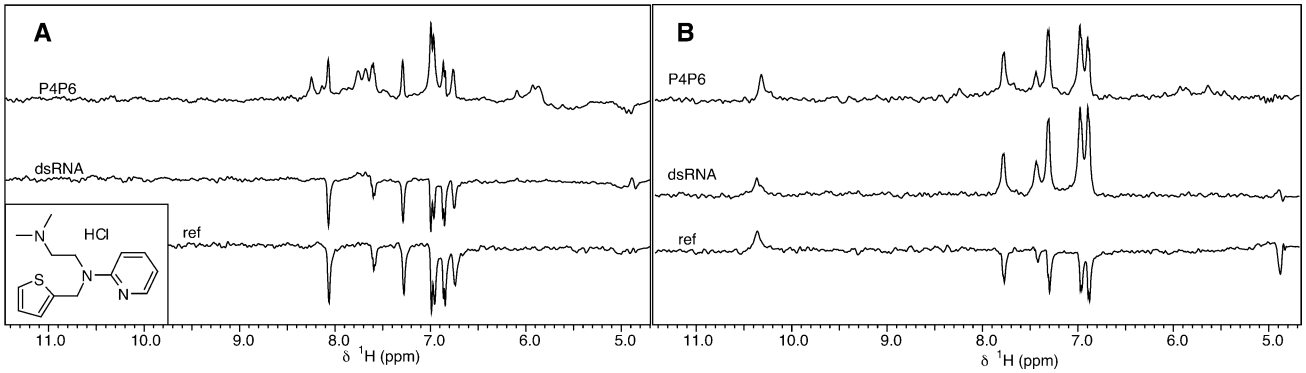

Figure 2. WaterLOGSY spectra of (A) the SHAPES specific binding compound (compound 53 − methapyrilene hydrochloride, shown in inset) and (B) a representative nonspecific binding compound (compound 65). Each of the above samples contained 1 mM ligand. The top traces were from samples containing 50 μM P4P6 RNA, and the middle traces were from samples containing 20 μM dsRNA. The larger size and more extended structure of dsRNA results in a longer correlation tumbling time as compared to P4P6, such that these concentrations of RNA should give similar WaterLOGSY signals.

to work for an RNA target,[9] we found that the STD method does not work particularly well for RNA screening, as described in detail previously.[4] It is likely that the approximately 2-fold lower proton density in RNA molecules, as compared to proteins, makes the spin diffusion of magnetization throughout the RNA much less efficient.

We therefore used the WaterLOGSY experiment to screen the SHAPES library. Screening the library as 28 mixtures with four compounds in each, we found that 23 of the SHAPES compounds interact with P4P6, 17 of which are positively charged.

One of the limitations of the ligand-based NMR screening methods as compared to those that directly observe the macromolecule resonances is the lack of information regarding the specificity of the interaction. If a specific binding site with known ligands is targeted, competition binding experiments can be used to identify which WaterLOGSY hits compete for the same binding site.[8] Instead, we have chosen to use a less stringent, but more general, measure of specificity. To identify those ligands that interact with RNA though nonspecific intercolation or electrostatic interactions, we have used a 136 base pair duplex A-form helical RNA (referred to as dsRNA) as a counterscreen. Because of the high concentrations of ligands used for the screen, any compounds that bind to dsRNA may still bind better to P4P6. However, this counterscreen provides a rapid way to identify those compounds that most strongly favor the binding of P4P6 over dsRNA.

For each of the 23 SHAPES compounds that bind P4P6, we recorded WaterLOGSY spectra in the presence of the dsRNA control. Of the 23 P4P6 hits, only compound 53, methapyrilene hydrochloride, bound specifically according to this criterion. Figure 2A shows the WaterLOGSY spectra of this compound for the reference sample, and the P4P6 and dsRNA control samples. The inversion of the WaterLOGSY signal in the presence of P4P6 is a clear indication of binding. The similar intensity negative signals for the reference sample and the sample containing the dsRNA control indicate that this compound is not interacting with the dsRNA control. For comparison, the WaterLOGSY spectra for a representative nonspecific binder, compound 65, are shown in Figure 2B. The positive WaterLOGSY peaks for this compound in the presence of both P4P6 and dsRNA clearly indicate that it is binding both RNAs.

This work demonstrates the feasibility of using NMR spectroscopy to screen a small focused library for binding to an RNA target. Although many of the technical issues relevant to using NMR with protein targets are similar to the issues faced when screening RNA targets, there are differences which must be considered. Of the three NMR methods used in this study, we found that the WaterLOGSY method was the most sensitive experiment for our RNA target.

In addition to considering the NMR experiments that are most appropriate for screening RNA targets, as we investigated in this study, the choice of screening libraries may be of equal importance. Because the interactions important for stabilizing a small molecule binding specifically to RNA may be different from the interactions important for the binding to a protein, the optimal screening library for an RNA target may be different from that for a protein target. The SHAPES library used in this study was designed and previously used for screening protein targets. Although we succeeded in identifying a specific RNA binding compound from this library, use of an RNA-directed screening library might result in improved hit rates. As more efforts focus on RNA as a therapeutic target, more knowledge will be generated to address this important issue.

Acknowledgment. This work has been supported by grants from the National Institutes of Health (GM-53320) and the Skaggs Institute for Chemical Biology, and Postdoctoral Fellowship Grant #PF-02-022-01-CDD to E.C.J. from the American Cancer Society.

References

(1) Peng, J.; Lepre, C.; Fejzo, J.; Abdul-Manan, N.; Moore, J. *Methods Enzymol.* **2001**, *338*, 202−230.
(2) Cheng, A. C.; Calabro, V.; Frankel, A. D. *Curr. Opin. Struct. Biol.* **2001**, *11*, 478−484.
(3) Fejzo, J.; Lepre, C. A.; Peng, J. W.; Bemis, G. W.; Ajay; Murcko, M. A.; Moore, J. M. *Chem. Biol.* **1999**, *6*, 755−769.
(4) Lepre, C. A.; Peng, J.; Fejzo, J.; Abdul-Manan, N.; Pocas, J.; Jacobs, M.; Xie, X.; Moore, J. M. *Comb. Chem. High Throughput Screening* **2002**, *5*, 583−590.
(5) Cate, J. H.; Gooding, A. R.; Podell, E.; Zhou, K.; Golden, B. L.; Kundrot, C. E.; Cech, T. R.; Doudna, J. A. *Science* **1996**, *273*, 1678−1685.
(6) Mayer, M.; Meyer, B. *Angew. Chem., Int. Ed.* **1999**, *38*, 1784−1788.
(7) Meyer, B.; Weimar, T.; Peters, T. *Eur. J. Biochem.* **1997**, *246*, 705−709.
(8) Dalvit, C.; Fogliatto, G.; Stewart, A.; Veronesi, M.; Stockman, B. *J. Biomol. NMR* **2001**, *21*, 349−359.
(9) Mayer, M.; James, T. L. *J. Am. Chem. Soc.* **2002**, *124*, 13376−13377.

JA037499S