J|A|C|S COMMUNICATIONS

Published on Web 02/27/2009

Stabilization of RNA Oligomers through Reverse Micelle Encapsulation

Hillary Workman and Peter F. Flynn*

Department of Chemistry, 315 South 1400 East Room 2020, University of Utah, Salt Lake City, Utah 84112

Received November 3, 2008; E-mail: peter.flynn@ utah.edu

The cellular milieu is rich in diversity of both simple and complex molecules, and also quite crowded. For example, in *E. coli*, the cytoplasm is estimated to contain 300−400 mg/mL of proteins, nucleic acids, and various small molecules.[1] It has become increasingly clear that both the average structure of a biological macromolecule and the time dependent changes in that structure depend in fundamental ways on the environment in which the molecule exists. The importance of these crowding effects for proteins has been recognized for some time,[2−5] but the equivalent analysis is largely unexplored in nucleic acids.

Structural fluctuations of RNA molecules are inherently linked to function in protein−RNA and RNA−RNA interactions, as well as RNA catalysis.[6−12] Previous in vitro studies have established that dynamics in RNA oligonucleotides can be measured and analyzed using biophysical tools such as FRET[13,14] and NMR relaxation methods.[12,15,16] The next major question is the extent to which crowding/confinement influences the structure and dynamics of the molecules. This question is central to a basic understanding of the behavior of RNA oligonucleotides and may also have important implications for drug discovery.

Encapsulation within surfactant-based reverse micelles has emerged as an effective biophysical tool. In this approach the molecule of interest is encased within a reverse micelle formed by amphiphilic molecules (surfactants) dissolved in low polarity bulk solvents.[17] The encapsulated RNA molecule can be reconstituted in solvent systems with varying bulk viscosities that can tune the rate of global tumbling to decouple motional modes from one another, and the oligomer can be reconstituted using a wide range of conditions that allow for investigation into the influence of hydration and salt effects. Finally, the area that may be most significant will be to explore the influence of crowding on the stability of RNA of oligonucleotides.

Here we describe the successful encapsulation of two previously characterized RNA oligonucleotides which are the TAR RNA from HIV and the 5′ stem loop oligonucleotide of the U4 snRNA (U4SL), using reverse micelles composed of CTAB with 1-hexanol as a cosurfactant. These two hairpin oligonucleotides are functionally important representatives of this common RNA structural motif. A comparison of the imino regions for free and encapsulated TetraTAR[12,18] RNA is shown in Figure 1. New resonances are observed for the encapsulated RNA relative to the free RNA, suggesting encapsulation may stabilize the RNA. Specifically, a new resonance at ∼13.2 ppm (Figure 1) is observed in the encapsulated state that does not appear in the spectrum of the free RNA. Based on assignments established for a related sequence,[19] this resonance corresponds to a uracil imino that is adjacent to an internal bulge. This suggests that encapsulation may stabilize the bulge region of TetraTAR RNA, as this uracil forms a U−A base pair to close the bulge on the 5′ side. In addition, a new resonance appears at ∼11.9 ppm that by analogy appears to correspond to the imino proton of a guanine residue near the terminus of the hairpin. The presence of this resonance is thus consistent with stabilization of the terminal stem region. The resonance at ∼10 ppm corresponds to a non-hydrogen bonded guanine imino proton resonance.

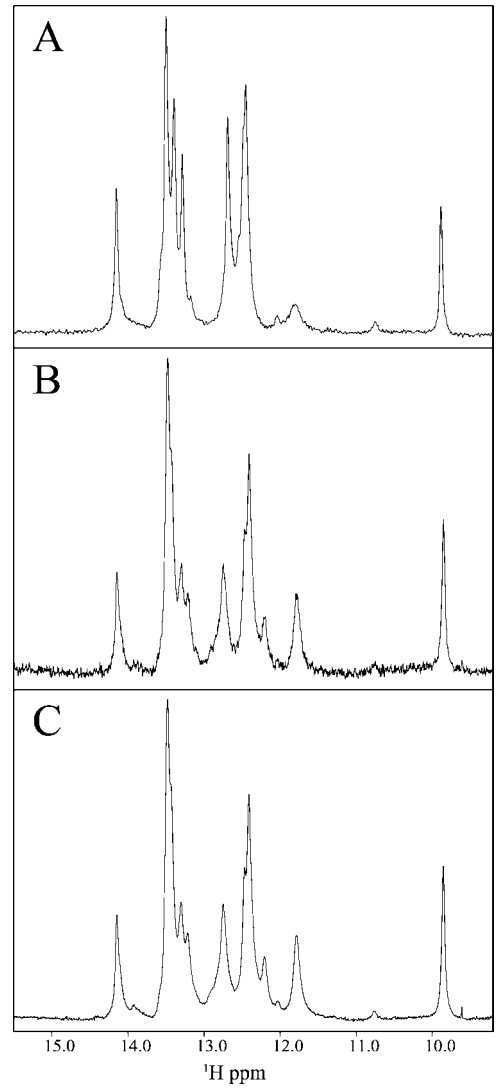

Figure 1. (A) Imino spectra of free TetraTAR in H_2O recorded with 64 transients. (B) TetraTAR encapsulated in CTAB reverse micelles recorded with 64 transients. (C) TetraTAR encapsulated in CTAB reverse micelles recorded with 1024 transients. CTAB reverse micelles contained 150 mM CTAB, 8% hexanol, and w_0 of 20. All data were obtained using the WET g11echo experiment recorded on a 600 MHz spectrometer at 10 °C.

Comparative spectra of encapsulated and free U4SL are shown in Figure S3. The ^{1}H NMR spectrum of encapsulated U4SL has a similar chemical shift profile to free U4SL in solution, but with the addition of several new peaks at 14.5, 11.9, 11.7, 11.1, and 10.1 ppm, consistent with significant stabilization of the oligonucleotide. Of particular interest is the appearance of the peak at

10.1021/ja8084753 CCC: $40.75

10.1 ppm. Peaks in this region are indicative of a sheared G·A base pair,[20] suggesting that encapsulation may have stabilized the base pairs present in the kinked structure.

The interior radii of the CTAB reverse micelles used in this study are ~25 Å (50 Å diameter), which in principle would be large enough to accommodate the fully extended forms of either the tetraTAR or U4SL oligonucleotides (~30 Å). Both the tetraTAR and U4SL oligonucleotides are believed to possess conformational flexibility,[12,14,21] and the results we have obtained indicate that confinement restricts the conformational range of these molecules. In comparing results of encapsulation on the two RNA oligonucleotide molecules, it is apparent that the imino resonances present in the tetraTAR RNA spectrum become slightly narrower upon encapsulation, while resonances present in the U4SL spectrum become somewhat broader. An important factor in the observed differences may be the effective correlation times for the imino protons for molecules free in solution versus those found for the encapsulated molecules. The tetraTAR RNA model oligonucleotide is a relatively stable RNA construct, as is clear from inspection of the imino region of the ^{1}H NMR spectrum; e.g., most of the anticipated imino resonances are observed. The local correlation time for imino protons in the TAR RNA oligonucleotide thus largely reflects the global correlation time of the entire molecule, which has been shown to be in the nanosecond time range.[12] Encapsulation in the CTAB/*n*-pentane system decreases the global correlation time, leading to a slight sharpening of the imino proton NMR resonances in the spectrum of the TetraTAR RNA molecule. By contrast, the U4SL construct has a relatively labile structure, wherein many of the imino ^{1}H NMR resonances anticipated based on crystallographic studies of the molecule are missing. The correlation time for imino protons present in the U4SL oligonucleotide are thus anticipated to contain a significant contribution from local motion. It appears that encapsulation damps out a portion of the local motion, which in turn increases the effective correlation time leading to a broadening of the imino proton NMR resonances for the U4SL sample.

Changes in resonance line widths may also be due in part to changes in the rate of imino proton exchange. According to the standard model, the rate of imino proton exchange depends on both the buffer employed to catalyze the exchange and motional coupling that exposes the labile imino proton to exchange. We anticipate that hydrogen exchange in encapsulated macromolecules is likely to be influenced by a generalized reduction in the amplitude of local motion[4] and by the additional structure present in the coencapsulated water molecules. Both of these influences will be present within the cell and, thus, represent important considerations for the analysis of structure and function of RNA oligonucleotides and their complexes.

In addition to changes in resonance line widths, it is apparent that the sensitivity of the encapsulated spectra is lower than the free solution counterparts. The lower sensitivity of the encapsulated sample is due to the limited amount of material available to conduct surveys of encapsulation conditions. Based on our experience with proteins, we are confident that complex RNA oligonucleotides of sizes relevant to structural biology and biophysics can be encapsulated at concentrations that are favorable to effective study.

RNA encapsulation within reverse micelles provides a new method for investigating RNA structure and dynamics by providing a confining environment for the target RNA molecule that may be similar in important respects to the cellular environment. Future development of this method should include a survey of surfactant/cosurfactant systems, water loading parameters, ionic strength conditions, and a thorough study of the effects of crowding and confinement on RNA structure and dynamics.

Acknowledgment. The authors acknowledge the support of a Seed Grant Award from the University of Utah Research Foundation. H.W. was supported by the Biological Chemistry Training Grant (GM 8537).

Supporting Information Available: The protocol for preparation of the encapsulated RNA oligonucleotides is described. Comparative spectra for the 5′ stem loop hairpin of the U4snRNA are also reported. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Zimmerman, S. B.; Trach, S. O. *J. Mol. Biol.* **1991**, *222*, 599–620.
(2) Ellis, R. J. *Curr. Opin. Struct. Biol.* **2001**, *11*, 114–119.
(3) Minton, A. P. *J. Pharm. Sci.* **2005**, *94*, 1668–1675.
(4) Simorellis, A. K.; Flynn, P. F. *J. Am. Chem. Soc.* **2006**, *128*, 9580–9581.
(5) Van den Berg, B.; Ellis, R. J.; Dobson, C. M. *EMBO J.* **1999**, *18*, 6927–6933.
(6) Draper, D. E. *J. Mol. Biol.* **1999**, *293*, 255–270.
(7) Höbartner, C.; Micura, R. *J. Mol. Biol.* **2003**, *325*, 421–431.
(8) Hoogstraten, C. G.; Wank, J. R.; Pardi, A. *Biochemistry* **2000**, *39*, 9951–9958.
(9) Madhani, H. D.; Guthrie, C. *Annu. Rev. Genet.* **1994**, *28*, 1–26.
(10) McManus, C. J.; Schwartz, M. L.; Butcher, S. E.; Brow, D. A. *RNA* **2007**, *13*, 2252–2265.
(11) Penedo, J. C.; Wilson, T. J.; Jayasena, S. D.; Khvorova, A.; Lilley, D. M. *RNA* **2004**, *10*, 880–888.
(12) Zhang, Q.; Sun, X.; Watt, E. D.; Al-Hashimi, H. M. *Science* **2006**, *311*, 653–656.
(13) Gohlke, C.; Murchieo, A.; Lilley, D. M. J.; Clegg, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11660–11664.
(14) Goody, T. A.; Melcher, S. E.; Norman, D. G.; Lilley, D. M. J. *RNA* **2004**, *10*, 254–264.
(15) Vallurupalli, P.; Kay, L. E. *J. Am. Chem. Soc.* **2005**, *127*, 6893–6901.
(16) Shajani, Z.; Varani, G. *J. Mol. Biol.* **2005**, *349*, 699–715.
(17) Flynn, P. F. *Prog. Nucl. Magn. Res. Spectrosc.* **2004**, *45*, 31–51.
(18) Fischer, A.; Gdaniec, Z.; Olejniczak, M.; Bielecki, L.; Adamiak, R. W. *Nucleic Acids Symp. Ser.* **1999**, *42*, 117–118.
(19) Kim, I.; Liu, C. W.; Puglisi, J. D. *J. Mol. Biol.* **2006**, *358*, 430–442.
(20) Flinders, J.; Dieckmann, T. *Prog. Nucl. Magn. Res. Spectrosc.* **2006**, *48*, 137–159.
(21) Vidovic, I.; Nottrott, S.; Hartmuth, K.; Lührmann, R.; Ficner, R. *Mol. Cell* **2000**, *6*, 1331–1342.

JA8084753