Rapid Compaction of an RNA Lacking Tertiary Contacts

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Introduction

Recently experiments have begun to probe the most rapid events in the folding of different RNAs (see [1] for a recent review of RNA folding). Our experiments have focused on studying the earliest events in the folding of a large ribozyme, the *Tetrahymena* group I intron, following the addition of divalent Mg ions. Our previous experiments showed that, under these conditions, this ribozyme collapses to a state that is nearly as compact as the native state within 1 second of the initiation of folding [2]. The observation of this rapid collapse was unexpected, given that stable native contacts are not observed to form during the first second of folding of this ribozyme [3]. Time-resolved small-angle x-ray scattering (SAXS) experiments indicated that compaction occurred in two separate phases, with time constants on the low-millisecond scale and 100-ms scale. This year, we performed experiments to probe the initial compaction of a mutant RNA in which the five predominant tertiary contacts have been removed by mutation.

Methods and Materials

We employ a microfabricated, rapid fluid mixing cell to trigger and monitor the shape change of RNA following the addition of 10 mM of Mg^{2+} to initiate tertiary structure formation. A schematic of the experimental apparatus is shown in Fig. 1; its operation is described in the figure caption. As in previous experiments [2], pink beam was employed at IMM-CAT at the APS [4]. All of these measurements were performed at beamline station 8-ID-I. The beam size is typically 10×40 μm.

Results

Folding of the wild-type and mutant ribozymes was probed under otherwise identical conditions (addition of 10 mM of Mg^{2+}). Individual scattering profiles acquired at different times are shown in Fig. 2. Folding progress was monitored as in previous work [2], by projecting the time-dependent profiles onto the initial and final states. Longer-time data (acquired by collaborators at beamline 12-ID and reported in Ref. [5]) provide the endpoints for the compaction phases.

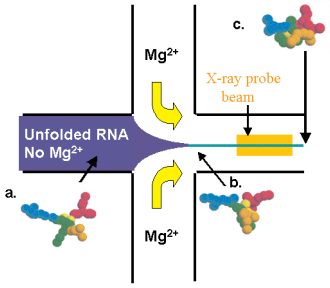

*FIG. 1. Cartoon of an RNA folding experiment. Folding is initiated within the microfabricated flow cell by the rapid addition of small Mg^{2+} ions by diffusion. The RNA **folds as it flows** down the channel; the x-ray beam can be moved to probe the conformation at any position in the flow cell. Schematic conformations [2] of the RNA are shown at different locations along the channel. Molecule **a** is unfolded, molecules **b** and **c** are captured during folding.*

Discussion

These results show that the kinetics of compaction are dramatically altered by the removal of the five key tertiary contacts in the *Tetrahymena* ribozyme [5]. Small changes are observed in the first phase, suggesting that it is largely due to electrostatic relaxation; time constants of 2.9 ±1.3 ms and 4.4 ±0.6 ms for the wild-type and mutant molecule, respectively, were determined from the data shown above. A second phase of compaction was not observed in the mutant, suggesting that it results from the transient formation of tertiary contacts within the RNA.

Acknowledgments

We acknowledge the valuable assistance of L. Lurio and H. Gibson with the x-ray experiments. This work was supported by the National Science Foundation (NSF) through the Cornell Nanobiotechnology Center and by the National Aeronautics and Space Administration (NASA) under NAG8-1778. These experiments made use of the Cornell Nanofabrication Facility, supported by the NSF, Cornell University, and industrial affiliates. This work was performed at IMM-

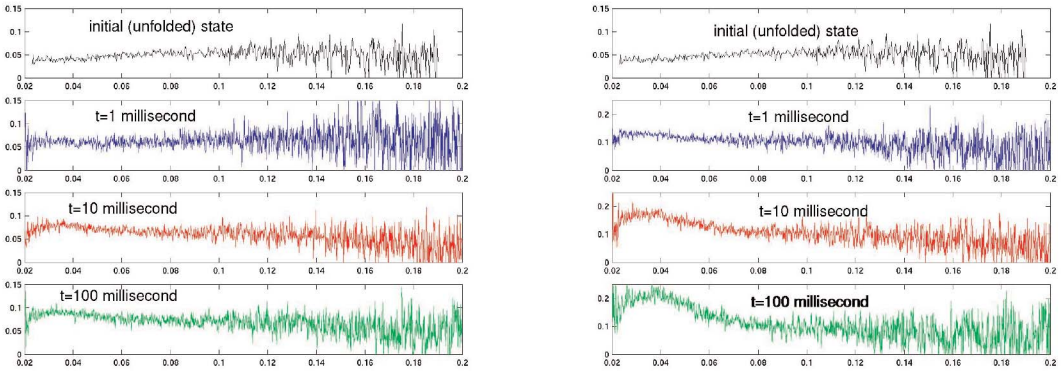

FIG. 2. Time-resolved Kratky plots ($I*q^2$ versus q, where $q = 4\pi\sin\theta/\lambda$) at four different times during folding. The left panel shows the evolution of scattering profiles for the mutant ribozyme; the right panel shows the evoluation of scattering profiles for the wild type. The compaction, assayed by the size of the low q peak, is much more pronounced in the wild-type RNA than in the mutant after 100 ms.

CAT beamline ID-8-I. Use of the APS was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-ENG-38.

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