Initial Folding Events of RNA

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Introduction

Like proteins, RNA can fold to functional three-dimensional structures. Therefore an analogous RNA folding problem exists to elucidate the relationship between nucleotide sequence and structure.[1] Indeed, because of the lower information content of the RNA chain, the folding problem might be more easily solved for RNA than for proteins. A recent study of tertiary structure formation of the *Tetrahymena* group I RNA by time-resolved small-angle x-ray scattering clearly shows significant structural rearrangement within the 1 minute dead time of those experiments.[2] We have initiated an effort to probe structural rearrangement of this ribozyme on timescales as short as milliseconds, using a continuous flow cell previously employed for protein folding studies.[3]

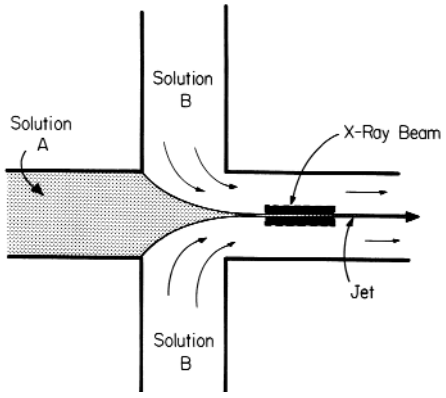

FIG. 1. A schematic of the flow cell.

Methods and Materials

We employ a microfabricated rapid fluid mixing cell to trigger and monitor the shape change of RNA on initiation of tertiary structure formation.[3,4] For these experiments RNA tertiary structure formation is triggered by the addition of Mg^{2+}. A schematic of the flow cell is shown in Fig. 1. Solution A contains 'unfolded' RNA (possessing secondary structure) in buffer. Solution B contains 10 mM Mg and is otherwise identical to Solution A.

As in previous work,[3] pink beam was employed at the IMMYT-Whitehead-CAT;[5] however these measurements were performed in the 8-ID-I hutch. Beam intensities of 10^{11} and 10^{12} photons per second were used.

Results

Changes in the shape of the RNA are evident on short time scales, indicating rapid folding events in this system. A full report of this work is presently being written up for publication.[4]

Discussion

Small-angle x-ray scattering (SAXS) provides global structural information about macromolecules in dilute solution. When employed in a time-resolved mode, it is a useful probe of the large-scale structural changes that accompany important conformation changes, such as chain compaction during folding. The experiments described in this report demonstrate an application of newly-developed rapid time-resolved SAXS technology to non-protein systems.

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