Secondary Structure Analysis of Catalytic RNAs

Dennis Aldea, Logan Norrell, Uli Müller

Department of Chemistry & Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California

Abstract

There is strong scientific evidence that in early forms of life RNA fulfilled the hereditary and catalytic roles corresponding to DNA and proteins in today’s life forms. Catalytic RNAs are called ribozymes.

RNA secondary structure formation is essential for RNA catalysis. A ribozyme’s secondary structure forms when some ribonucleotides base pair with other ribonucleotides. Unpaired ribonucleotides are flexible and are readily altered by specific chemicals.

In this investigation, the secondary structure of R5_5C1, a triphosphorylation ribozyme discovered by the Müller lab1, was analyzed using selective 2'-hydroxyl acylation and primer extension (SHAPE)2. The Mfold RNA folding program was used to construct a possible secondary structure based on the SHAPE results3.

Triphosphorylation ribozymes attach a triphosphate group to a ribonucleotide, using trimetaphosphate (Tmp). Tmp is a prebiotically plausible molecule because it can be generated from phosphites4, which appear to have existed at 0.1 mM concentration in parts of the prebiotic ocean5. The Müller lab is interested in triphosphorylation ribozymes because they could provide an RNA world organism with chemical energy.

Selective Acylation

During summer research, various DNA templates that coded for ribozyme clone R5_5C1 were amplified via PCR and transcribed in vitro. The resulting RNA was purified by denaturing polyacrylamide gel electrophoresis (PAGE). The RNA was then selectively acylated by 1M7, a ribozyme that triphosphorylates RNA 5’-hydroxyl groups. Only flexible nucleotides are acylated by 1M7. 1M7 reacts with flexible ribonucleotides.

Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) is able to determine the length of each DNA fragment with single-nucleotide resolution. The gel image below was generated by a phosphomager, which visualizes the β-radiation emitted onto a screen by the radiolabeled primer.

For each nucleotide, a signal strength was calculated by subtracting the intensity of its control band from its experimental band. The values obtained from multiple experiments were scaled to form the continuous series shown above.

Further Research

Various experiments are needed to investigate whether the secondary structure constructed by the Mfold software is accurate. Such experiments could switch two paired nucleotides (base covariation) or remove an unnecessary section of the ribozyme (truncation).