Probing of the secondary structure of maxizymes

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ABSTRACT
The protein encoded by chimeric BCR-ABL mRNA causes chronic myelogenous leukemia (CML). We showed previously that a novel allosterically controllable ribozyme, of the type known as a maxizyme, can cleave this mRNA, with high specificity and high-level activity in vivo. In order to probe the putative conformational changes, we used a weakly alkaline solution to hydrolyze differentially phosphodiester bonds that were located in different environments. As indicated by earlier data obtained in vivo, our results demonstrated that the active conformation was achieved only in the presence of the junction within the chimeric BCR-ABL mRNA.

INTRODUCTION
RNA molecules fold into well-defined structures to perform biological and chemical functions. Folded structures of RNA contain regions of double-stranded duplex, hairpins, internal loops, bulged bases and pseudoknotted structures. Knowledge of the structure and general rules for RNA folding will be valuable to infer a more detailed mechanism of RNA function.

We recently succeeded in designing a novel maxizyme that can specifically cleave BCR-ABL mRNA upon formation of a dimeric structure, in which one of the two substrate-binding regions of the heterodimer serves as sensor arms (1). Since the junction sequence in the BCR-ABL mRNA has a critical effect on the catalytic activity of the maxizyme, we can assume that it plays an important role in maintaining the active structure (Fig. 1). In order to confirm that the proposed conformational changes actually occur, we developed a novel chemical probing procedure using a weakly alkaline solution (pH 9.2) in the active form

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<tr>
<th>Substrate</th>
<th>5'CCUCAGGGUCUGAGUG 3'</th>
<th>A</th>
<th>C</th>
<th>U</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCR-ABL activator</td>
<td>3'GGAGUCCCA ACUCAC 5'</td>
<td>A</td>
<td>C</td>
<td>U</td>
<td>G</td>
</tr>
<tr>
<td>MzL U-A MzR</td>
<td>3'CUCCGAA GAAGGAUAACUACACAG 5'</td>
<td>A</td>
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<td>U</td>
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In inactive form

<table>
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<tr>
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Figure 1. Three types of putative structures mediated by different effectors. (A) In the presence of BCR-ABL mRNA. (B) In the absence of BCR-ABL mRNA. (C) In the presence of ABL mRNA.
presence of 25 mM Mg\textsuperscript{2+} ions. To our knowledge, our novel method is the first method for the analysis of the secondary structure of functional RNAs that simply uses a weakly alkaline solution for hydrolysis.

RESULTS AND DISCUSSION

It is well known that, at high pH, all phosphodiester bonds in RNAs are cleaved rapidly because of intramolecular anchimeric attack by 2'-OH groups. By contrast, in weakly alkaline solution, we anticipated that rates of hydrolysis of phosphodiester bonds in duplex region might be lower, because of the rigid conformation (2), than corresponding rates in single-stranded regions, such as hairpin and internal loops. In view of the probable advantages of the chemical probing of functional RNAs by simple weak-alkaline hydrolysis, we attempted to perform such analysis using our maxizyme.

In our experiments, either MzR or MzL was separately end-labeled at the 5' end with \textsuperscript{32}P. Non-\textsuperscript{32}P-labeled RNA was added in molar excess. The phosphodiester bonds that were hydrolyzed most rapidly were located in the predicted active core (internal loop region), and the phosphodiester bonds that we expected to be within duplex arms were significantly more resistant to alkaline hydrolysis. In order to confirm the results obtained with weakly alkaline solution, we performed an analysis using RNase T1. The analysis by weak alkaline hydrolysis and digestion with RNase T1 confirmed that, only in the presence of the BCR-ABL activator and Mg\textsuperscript{2+} ions, was the maxizyme capable of achieving an active conformation.

The discovery of the catalytic activity of certain RNAs (3,4) was followed by serious attempts to understand the mechanisms of assembly of such RNAs into functional molecules from linear strands. It has become increasingly apparent that the folding kinetics of RNA are complex, involving parallel pathways and kinetic traps. It is now very clear that BCR-ABL mRNA has a major structural effect on the maxizyme, as confirmed by the present chemical probing analysis and previous examinations in vitro and in vivo of the activity of the maxizyme (1).

In the absence of the BCR-ABL activator, the maxizyme appears to be in an open state and lacks sufficient energy to overcome the energy barrier required for activity. BCR-ABL mRNA binds both the sensor arms of MzR and MzL quite efficiently, facilitating the conversion of the open complex to the closed complex of the maxizyme. Then the catalytic core becomes more tightly packed with formation of the interface, and the energy cost for rearrangement of the conformation is paid by the binding of the activator. As a result, the inactive maxizyme becomes active.

All ribozyme-catalyzed reactions either require or are greatly stimulated by divalent metal ions, and these metal ions must function either catalytically, structurally or both. As demonstrated herein, it was only in the presence of Mg\textsuperscript{2+} ions (in addition to the BCR-ABL activator) that the maxizyme was converted from an inactive form to an active form, demonstrating the structural importance of the Mg\textsuperscript{2+} ions. In the presence of Mg\textsuperscript{2+} ions, the maxizyme undergoes structural changes, as do allosteric proteinaceous enzymes, depending on the presence or absence of an activator (BCR-ABL mRNA) or an inhibitor (ABL mRNA).

In our experiments in vitro and in vivo, the maxizyme cleaved the substrate only in the presence of BCR-ABL mRNA, without damage to normal transcripts and normal cells. Our chemical and enzymatic probing revealed that the intact maxizyme really does, in fact, adopt the expected active structure. The simple method for probing RNAs with weak alkali seems to be as useful as methods that involve more specific cleavage of specific bonds. The various features of the maxizyme should provide a firm basis for the design of novel ribozymes for gene therapy (1,5,6).

REFERENCES