The Synthesis of Ribozymes using TC-RNA Chemistry

Steena Athan, Ulrich Isaks, Grant McGeech, Catherine McKeen and Douglas Pickren; Link Technologies Ltd, Bletchley, UK.
Salina Möller and Anne Schraut; Institut für Biochemie, Bioraupharm Chemie, Ernst Moritz Arndt Universität Greifswald, Germany.
catherine@linktech.co.uk

Introduction

Recently, 2'-O-(2-thioribose)-4-carboxylic acid (TC) was introduced as an alternative protection chemistry to TBOCS in RNA synthesis.1 This utilises a one step simple deprotection method that removes both the 2'-O- and nucleotide protection at the same time. The 2'-TC protecting group has been designed such that it deprotects at a slower rate than the carbohydrates, reducing the possibility of isomerisation during deprotection. Combined with the fact that the coupling efficiency is comparable with DNA synthesis, there is the potential to synthesise good quality long RNA such as ribozymes.

The synthesis of two ribozymes (55mer and 77mer) was carried out using TC-RNA phosphoramidite chemistry. Analysis of these was then carried out by MALDI MS, LCMS, and CGE. These were then compared to the same sequences synthesised via the more conventional TBOCS chemistry. The cleavage and ligation activity of both sets of oligonucleotides were then evaluated.

Experimental

Synthesis Conditions - TC RNA²

All oligonucleotides were synthesised on an ABI 394 DNA/RNA synthesiser with 5mm coupling and a 10min cap step. The amides were used at 0.51mM concentrations in toluene with 0.5M ETT in NMM as the activator. Molecular sieves (4Å) were used in both the activator and the amides. All supports used were DCC CLP.

Deprotection Conditions - TC RNA²

All oligomers were first treated with 20% DEA in MeOH. Deprotection was carried out with ethylene diamine/vehicle 1:1 at 54°C and the oligonucleotides eluted from the resin with 0.1M TEA.

Analysis Conditions - TC & TBOCS RNA

Crude oligonucleotides synthesised with TC chemistry were analysed by LCMS using an Agilent 12000 HPLC, equipped with a 6520 QTOF MS. Dr Zoltan Tamir, Agilent Technologies, Inc, B preceded this data. All purified oligonucleotides were analysed by ESI on a Bruker Ultraflex TOF on a Bruker Ultraflex. This data was provided by EGT-SA.

Ribozyme Cleavage Conditions

The substrate HPAS3 (TBOCS) was labelled with ATTO640, which is necessary to enable analysis with a LC-MS 4200I sequence. The following protocol was used ribozyme 500mM substrate, HPAS3 (25µM) TRIS-CH (pH 7.5) 300mM MgCl2, 100mM DMSO 40µg. Ribozymes, substrate, buffer and water were mixed and denatured at 95°C (2min), followed by incubation at 37°C (2min). The reaction was started by the addition of MgCl2 at 1:0. After 2, 4, 6, 8, 10, 20, 30, 60, 90 and 150min, aliquots of 1µl of the reaction mixture were added to 2μl stop mix (7°C, uren, 0.1% EDTA). Analysis was carried out on the sequence.

Results & Discussion

Oligonucleotide Synthesis

The oligonucleotide sequences synthesised for the 55mer and 77mer are shown in Figure 12. A 100mer sequence (although not used in any application) was also synthesised. To ensure that the synthesis was reproducible, three each of the 77mers and 55mers were made. These were analysed by LCMS and the results are summarised in Table 1, opposite, and Figure 12.

Table 1. Summary of LCMS analysis on the crude oligos

<table>
<thead>
<tr>
<th>Description</th>
<th>FL concn</th>
<th>Cycle yield</th>
<th>Expected Mw (Mass/average)</th>
<th>Found Mw (Average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA 55mer 191%</td>
<td>0.97%</td>
<td>(790.49)</td>
<td>(790.37)</td>
<td></td>
</tr>
<tr>
<td>RNA 55mer 27%</td>
<td>97.7%</td>
<td>(790.49)</td>
<td>(790.37)</td>
<td></td>
</tr>
<tr>
<td>RNA 55mer 31%</td>
<td>97.5%</td>
<td>(790.49)</td>
<td>(790.37)</td>
<td></td>
</tr>
<tr>
<td>RNA 77mer 28.2%</td>
<td>98.4%</td>
<td>(25048.45)</td>
<td>(25048.46)</td>
<td></td>
</tr>
<tr>
<td>RNA 77mer 35.6%</td>
<td>98.4%</td>
<td>(25048.45)</td>
<td>(25048.46)</td>
<td></td>
</tr>
<tr>
<td>RNA 77mer 25.4%</td>
<td>96.4%</td>
<td>(25048.45)</td>
<td>(25048.46)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 5. CGE of 55mer-TC after PAGE purification.

Figure 6. MALDI MS of 55mer-TC after PAGE purification.

Figure 7. CGE of 77mer-TC after PAGE purification.

Figure 8. MALDI MS of 77mer-TC after PAGE purification.

Figure 9. CGE of 77mer-TC after PAGE purification.

Figure 10. MALDI MS of 77mer-TC after PAGE purification.

Figure 11. CGE of 77mer-TC after PAGE purification.

Figure 12. MALDI MS of 77mer-TC after PAGE purification.

Riboyme Cleavage

The sequence shown in Figure 12 has a 3’ terminal 5’-TACGGTTAC-3’ and an internal 5’-TACGGTTAC-3’. This sequence is also present in the 77mer.

One 77mer oligonucleotide and one 55mer oligonucleotide were PAGE purified and analyzed by MALDI TOF and CGE. The results are shown opposite in Table 2. The most significant difference is with the 77mer TC when 77mer-TC has 36% higher purity than 77mer-TBOCS.

Conclusions

TC chemistry provides a means of synthesising long RNA oligonucleotides with comparable yields to TBOCS chemistry; but with higher purity. Some work still needs to be carried out to optimise the deprotection time in order to avoid incomplete deprotection.

Further Information

Any queries regarding this work should be directed to Dr Catherine McKeen, Product Manager, Link Technologies Ltd. www.linktech.co.uk