Towards DNA based esterases: uncovering Hoogsteen face regulation of the pKa_H of a tethered imidazole functionality by NMR and molecular dynamics

Dieter Buyst1,2, Vicky Gheerardijn2, Bjorn Van Gasse1, Jos Van den Begin2, Annemieke Madder2 & José C. Martins3

1 NMR, Department of Organic Chemistry, University of Ghent, Krijgslaan 281 S4, B-9000, Ghent, Belgium
2 OBCR, Department of Organic Chemistry, University of Ghent, Krijgslaan 281 S4, B-9000, Ghent, Belgium (*e-mail: Dieter.Buyst@UGent.be)

1. General concept & design

Template sequence = Wild Type

5'-GACCATCTGCAGCG-3'
3'-CTGGTAAAACGTGCC-5'

In the development of esterase like DNAzymes, recent advances in OBCR made the introduction of amino acid-like side chain functionalities on the thymine base possible. The hydrophobic and chiral environment of a B-DNA helix is used as a new scaffold for the development of synthetic enzymes. Both first generation, single histidine modified systems and second generation, double modified systems are subjected to a systematic study.

2. First glimpse of the pKa_H regulating motif

Thermal stability

<table>
<thead>
<tr>
<th>System</th>
<th>WT</th>
<th>T1^H1</th>
<th>T2^H1</th>
<th>A-β base pair</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A-H2A-H3</td>
</tr>
<tr>
<td>Tm (°C)</td>
<td>59.0 ± 0.1</td>
<td>65.0 ± 0.4</td>
<td>63.5 ± 0.2</td>
<td>+6.0 ± 0.3</td>
</tr>
<tr>
<td>Δ to WT</td>
<td>-</td>
<td>+6.0</td>
<td>+6.9</td>
<td></td>
</tr>
</tbody>
</table>

From melting temperatures:
- No loss of stability due to modification
- Increase in stability is dependent on position of modification: T1^H1 (+ 5-6°C)

4. Application of the motif in double modified systems

- Increase in melting temperature is consistent with presence of the pKa_H regulating motif for T1^H1 in both systems
- Increase in melting temperature is less pronounced with increasing vicinity of the imidazole functionalities

50ns of MD trajectory shows persistent H-bridging in T1^H1 and close interproton contacts validated in nOe's

5' -GACCATCTGCAGCG-3'
3'-CTGGTAAAACGTGCC-5'

In both systems the T1^H1 functionality shows an increased pKa_H value due to the intact motif

For the other T1^H1 functionalities (T2 or T1^H2) pKa_H decreases with increasing proximity due to repulsive positive charge interactions

References

Acknowledgements
The 700MHz is part of the INMR jointly operated by UGent, UA and VUB.