The Danish National Research Foundation and Danish Research Agency are thanked for financial support. Ms Britta M. Dahl, Department of Chemistry, University of Copenhagen, is thanked for oligonucleotide synthesis and Dr Michael Meldgaard, Exiqon A/S, for MALDI-MS analyses.

We have previously shown that xylo-configured oligonucleotides (XNAs, xylo nucleic acid) are able to form higher-order complexes, most likely triplexes, with single stranded DNA and RNA targets with increased thermal stability of the complexes formed [1] relative to the corresponding DNA reference. We have previously shown that the complexes formed between DNA reference and the DNA and RNA complements are of the duplex type under the conditions applied. 1 On the contrary, XNAs ON2 and ON3 do not display high-affinity recognition of RNA over DNA. The same tendency in increasing of thermal stability is observed in complex formation between xylo-clamp ON4 and XNA and LNA complements. We have previously shown that the complexes formed between DNA reference ON1 and the DNA and RNA complements are of the triple type under the conditions applied. 1 (On the contrary, XNAs ON2 and ON3 form XNA:DNA triplexes and also higher-order complexes with RNA, most probably triplexes of the same type.) We anticipate that the complexes formed with the xylo-clamp ON4 involves a bisemisotropic triplex and that the increased thermal stabilities, relative to XNA ON2, originate from relatively more favorable entropic changes during complex formation.

The results of thermal denaturation experiments are shown in Table 2. The almost fully modified homo-thymine XNA ON2 displays high-affinity recognition of natural RNA homo-adenine target in comparison with natural DNA. ON2 binds to the almost fully modified homo-adenine XNA complement less efficiently than with the RNA target while no complex formation between DNA ON4 and the almost fully modified homo-adenine XNA complement was observed. The modification of the DNA complement by introduction of seven conformationally locked adenosine LNA-type nucleotide units (LNA complement) instead of natural DNA units gave rise to a further increase in the thermal stability of the complexes formed with both the DNA reference ON1 and the almost fully modified homodyrimine XNA ON2. Modification of ON2 by the introduction of four conformationally locked 2′-amino-2′-deoxy-2′,3′,4′-trimethylene)xylofuranose nucleotide units (2′-amino-xylo-LNA units) instead of 2′-deoxy-β-D-xylofuranose units in the middle of the strand (XNA ON3) led to the formation of complexes with further increased thermal stabilities. For further optimization of binding properties of XNAs we have investigated xylo-clamp ON4. The data shown in Table 2 indicate that xylo-clamp ON4 binds with DNA and RNA complements more efficiently than XNA ON2. It should be noted that XNA ON4 in contrast to XNAs ON2 and ON3 do not display high-affinity recognition of RNA over DNA. The same tendency in increasing of thermal stability is observed in complex formation between xylo-clamp ON4 and XNA and LNA complements.

In present study we have investigated further the potential of XNAs as triplex forming oligonucleotides (TFOs) in an XNA:XNA:Target stoichiometry. In order to investigate triplex formation of xylo-configured TFOs with natural and modified targets we have prepared ON1-ON4 (shown in Table 1) on an automated DNA synthesizer using the standard phosphoramidite approach.

The hybridization data – Triplex (XNA:XNA:Target) formation [1]

<table>
<thead>
<tr>
<th>[d(A14)]</th>
<th>[r(A14)]</th>
<th>[dxT(AX)]</th>
<th>[dxTm(A)]</th>
<th>[La(A)]</th>
</tr>
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<tbody>
<tr>
<td>Tm, °C</td>
<td>Tm, °C</td>
<td>Tm, °C</td>
<td>Tm, °C</td>
<td></td>
</tr>
<tr>
<td>ON1</td>
<td>31</td>
<td>28</td>
<td>a</td>
<td>38</td>
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</tr>
<tr>
<td>ON3</td>
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<td>46</td>
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<td>n.d.</td>
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<td>ON4</td>
<td>43</td>
<td>42</td>
<td>10 and 35</td>
<td>53</td>
</tr>
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</table>

Acknowledgments

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References