Hexose nucleic acids

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Abstract - This manuscript is, in fact, an extended abstract, supplemented by self-explanatory figures (slides) presented at the lecture.

Why did Nature choose pentoses and not hexoses as sugar building blocks in her nucleic acids? Since the potential for constitutional self-assembly for hexoses is comparable to that of pentoses, Nature's choice of pentoses must have had functional reasons. The question can be dealt with experimentally by synthesizing hexose analogues of natural nucleic acids, studying their chemical properties and systematically comparing these properties with those of their natural counterparts. Differences in such properties can be expected to reflect reasons why pentose nucleic acids are - as is implicit in the fact of their existence - superior to hexose alternatives with respect to biological function.

The lecture summarizes first the results of an experimental study on the pairing properties of synthetic oligonucleotides that contain 2,3-dideoxy-glucopyranose in place of 2-deoxy-ribofuranose as sugar building block. This structure type ('homo-DNA') is a highly efficient autonomous pairing system with a pairing behaviour that is in part similar to, but also in part strikingly different from, the pairing behaviour of DNA.

The 2,3-dideoxy-hexopyranose building blocks of homo-DNA possesses - according to the criterion of a structure's potential for constitutional self-assembly - a more complex structure than the hexopyranose sugars of the (CH2)6 family. In contrast to the latter, 2,3-dideoxy-glucose should not be considered to belong to the group of potentially prebiological sugars and, therefore, the investigation of the chemistry of homo-DNA is only a model study for an exploration of the pairing properties of oligonucleotides derived from fully hydroxylated hexose sugars. Such studies are being carried out with both allolose and altrose - the two hexoses which constitute the main components in glycolaldehyde phosphate aldolization mixtures - as well as with glucose as the building blocks of hexopyranosyl-(4'→6')-oligonucleotides. The lecture describes the present state of these studies. Established already is the fact, that the pairing behaviour of such systems can be similar to, as well as drastically different from, the one shown by homo-DNA.

A comprehensive experimental involvement in the problem of a chemical rationalization of the natural nucleic acid's structure would require an extension of the study to hexopyranosyl- as well as to hexofuranosyl-oligonucleotide systems which have their phosphodiester link between positions other than the (4'→6') link of the systems investigated so far. Conformational analysis of such structures predicts the existence of a variety of potential pairing systems and, most interestingly, also foresees the existence of a ribopyranosyl isomer of RNA endowed with pairing properties akin to those of homo-DNA. Experiments towards a synthesis of such a "Pyranosyl-RNA" are in progress.
Homo-DNA: Summary of Experimental Observations

- Homo-DNA oligonucleotides form antiparallel purine-pyrimidine duplexes which are more stable than the corresponding DNA duplexes.

- The higher thermodynamic stability of Homo-DNA versus DNA duplexes is due not to greater binding energy, but rather to a less negative entropy of duplex formation.

- In Homo-DNA adenine and guanine pair strongly with themselves: the base pairing selectivities in Homo-DNA are different from those operating in DNA.

- In Homo-DNA guanine/iso-guanine and xanthine/2,6-diaminopurine form base pairs which are comparable in strength to guanine/cytosine.

- Complementary base sequences of Homo-DNA and DNA do not pair: Homo-DNA is an autonomous artificial pairing system.

MELTING TEMPERATURES (°C) OF HEXAMER - DUPLEXES
15 - 20 μM Nucleotide, 150 mM NaCl, 10 mM Tris pH 7

<table>
<thead>
<tr>
<th>Homo-DNA</th>
<th>DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADENINE</td>
<td>A   -</td>
</tr>
<tr>
<td>THYMINE</td>
<td>T &lt; 5</td>
</tr>
<tr>
<td>GUANINE</td>
<td>G - 38</td>
</tr>
<tr>
<td>CYTOSINE</td>
<td>C - 58</td>
</tr>
</tbody>
</table>

MARKUS BOEHINGER, HANS-JÖRG ROTH, JÖRG HUNZIKER, FREDI GIGER, DR. CHRISTIAN LEUMANN

Thermodynamic Data of Homo-DNA(=dc) and DNA(=d)-Oligonucleotide Duplexation

\[
\begin{align*}
\Delta G^\circ & = -10.5 \text{ kcal/mol} \\
\Delta H & = -44.0 \text{ kcal/mol} \\
T_m & = 55°C
\end{align*}
\]

\[
\begin{align*}
\text{dA}_2T_5 & \quad \Delta G^\circ = -7.1 \text{ kcal/mol} \\
\text{ddA}_2T_5 & \quad \Delta G^\circ = -10.5 \text{ kcal/mol}
\end{align*}
\]

\[
\begin{align*}
\text{dA}_2T_5 & \quad \Delta H = -13.5 \text{ kcal/mol} \\
\text{ddA}_2T_5 & \quad \Delta H = -10.5 \text{ kcal/mol}
\end{align*}
\]

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HOMO-DNA: TOPOLOGIES OF ADENINE–ADENINE PAIRING

WATSON–CRICK

REVERSE WATSON–CRICK

HOOGSTEEEN

REVERSE HOOGSTEEEN

GUANINE–GUANINE PAIRING

HOMO-DNA:

ANTIPARALLEL

ANTI–ANTIPARALLEL

QUASI–ISOMORPHOUS
WITH A/A–HOOGSTEEEN–PAIRING

SYN–ANTI (OR
ANTI–SYN
ANTI–PARALLEL

3 H C E N + N E C – C E N

NH₃

H₂O

H₂O

H₂O

H₂O

2,6-DIAMINOPURINE

GUANINE

ISOGUANINE

XANTHINE

H₂O (–NH₃)

GUANINE–ISOGUANINE

2,6-DIAMINOPURINE–XANTHINE

HOMO-DNA:

PURINE–PURINE PAIRING

MELTING TEMPERATURES (°C) OF HEXAMER–DUPLEXES:

15-20µM Nucleotide
150mM NaCl
10mM Tris pH 7

<table>
<thead>
<tr>
<th>Adenine</th>
<th>A</th>
<th>47</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxanthine</td>
<td>H</td>
<td>&lt;7</td>
</tr>
<tr>
<td>2,6-Diaminopurine</td>
<td>D</td>
<td>41 &lt;5</td>
</tr>
<tr>
<td>Xanthine</td>
<td>X</td>
<td>14</td>
</tr>
<tr>
<td>Isoxanthine</td>
<td>I</td>
<td>43</td>
</tr>
<tr>
<td>Guanine</td>
<td>G</td>
<td>15</td>
</tr>
</tbody>
</table>

KATRIN GROEBKE, MARKUS BOEHNING, HANS-JÖRG ROTH, JÖRG HUNZKER, ULF DIEIDERICHSEN, DR. WILLIAM FRASER, DR. CHRISTIAN LEUMANN
ALLOSE-NA: Summary of Experimental Observations

- Purine-pyrimidine pairing in allopuranosyl oligonucleotides (up to dodecamers) is very much weaker, and almost certainly of different constitutional type, than in either homo-DNA or DNA.

- Adenine pairs with adenine, and guanine with guanine, as in homo-DNA, but more weakly.

- Guanine and isoguanine pair strongly, as in homo-DNA. However, in contrast to homo-DNA, the pairing is heavily dependent on base-pair sequence.

- There appears to be no guanine-cytosine cross-pairing to homo-DNA.
DNA (RNA)  
HOMO-DNA  
ALLO-PYRANOXYL-NA  
ALTRO-PYRANOXYL-NA  

BASE PAIRING:
- SELECTIVE
- INDEPENDENT OF pH
- QUASI-INDEPENDENT OF BASE-PAIR SEQUENCE
- SELECTIVE BUT MORE PROLIFERATED THAN DNA
- INDEPENDENT OF pH
- QUASI-INDEPENDENT OF BASE-PAIR SEQUENCE
- MUCH WEAKER THAN HOMO-DNA
- DEPENDENT ON pH
- STRONGLY DEPENDENT ON BASE-PAIR SEQUENCE
- STRONGER THAN ALLO-PYRANOXYL-NA
- WEAKER THAN HOMO-DNA
- BUT (PRESUMABLY) STRUCTURALLY SIMILAR

Glucose-NA: Gloc-(AAAAAAUUUUU) does not pair (according to UV-spectroscopy)

Constitutionally Isomeric Oligonucleotide Backbones
(Phosphodiester junctions between sugar positions)

<table>
<thead>
<tr>
<th>Sugar Type</th>
<th>2'→6'</th>
<th>2'→4'</th>
<th>3'→6'</th>
<th>3'→4'</th>
<th>3'→5'</th>
<th>4'→6'</th>
</tr>
</thead>
<tbody>
<tr>
<td>PYRANOSES</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>FURANOSES</td>
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<td></td>
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<tr>
<td>HEXO-</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>PENTO-</td>
<td></td>
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</tbody>
</table>

- retrosynthetically derivable via aldolization pathway
- cooperative base pairing predicted by qualitative conformation analysis
- experimentally studied (so far)

U. Diederichsen
β-RIBO-PYRANOSYL-(2’→4’)-OLIGONUCLEOTIDES
("PYRANOSYL - RNA")
Pairing Conformation of Backbone (hypothetical)

- 2 · 3⁴ (idealised) backbone conformations
- 10 of them least strained (gg- as well as gt-phosphodiester groups)
- only 1 conformationally repetitive (gt)

β-RIBO-PYRANOSYL-(2’→4’)-OLIGONUCLEOTIDES
("PYRANOSYL - RNA")
a target of chemical synthesis and of studies on the constitutional self-assembly of potentially self-replicating systems:

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