Solution Equilibria of the i-motif-forming Region Upstream of the B-Cell Lymphoma-2 P1 Promoter

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Outline

- Introduction
  - The G-quadruplex at the *bcl-2* promoter site
  - The cytosine-rich complementary strand
- Dealing with spectroscopic multivariate data
- Results
  - Acid-base properties
  - Melting behavior
  - A possible structure for the i-motif
  - Interaction with a porphyrin: TmPyP4
The *bcl-2* oncogene

*Bcl-2* gene has been classified as a proto-oncogene because of its overexpression in a wide range of human cancers.

*Bcl-2* gene product is a protein involved in the control of programmed cell death *(apoptosis)*.

The oncogenic potential of *bcl-2* is achieved by *reducing the rate of cell death*.

*Bcl-2* has two promoters:

The GC-rich region upstream of the P1 promoter has been shown to be critically involved in the *regulation* of *bcl-2* gene expression.
Dexheimer et al. have shown that the guanine-rich strand of the DNA in this region can form three *intramolecular* G-quadruplex structures.

The central G-quadruplex, which is the most stable, forms a mixed *parallel/antiparallel* structure:
Why the study of the cytosine-rich sequence?

The sequence 5’-GGGCAGCGGGAGGAAGGGGGGCGGG-3’ has shown to form a G-quadruplex...

... however, this sequence is not isolated in vivo, and the complementary C-rich strand is also present.

C-rich strands can form stable structures known as i-motifs:

Protonation at N3 is required!

Therefore… duplex? formation of G-quadruplex and i-motifs? mixtures?
Objectives of our work

We want to know the solution equilibria of the sequence:

5’CCC GCC CCC TTC CTC CCG CGC CCG-3’

corresponding to the middle region of the bcl-2 NHE region

“Solution equilibria”:

- acid-base properties of this sequence…
  - in which pH range the i-motif is formed?
  - is there more than one i-motif?

- thermal denaturation

- interaction with a G-quadruplex-binding ligand: TmPyP4
Molecular absorption-monitored acid-base titration

pK\text{a} of cytosines is around 4.5, depending on ionic strength

Therefore, absorbance traces seem to point out to the formation of i-motif at pH < 7
NMR-monitored acid-base titration

The signal around 13.2 ppm is characteristic of guanine imino protons involved in Watson-Crick base pairs.

The signal around 15.6 ppm indicates the presence of imino H3C+ protons.

The signal around 13.2 ppm is characteristic of guanine imino protons involved in Watson-Crick base pairs.
CD-monitored acid-base titration

Formation of i-motif at pH < 7 is reflected around 290 nm

At least, two protonation steps are observed

It is difficult to ascertain the presence of more than one i-motif!

A possible tool: multivariate data analysis
The well-known Beer-Lambert law for a single wavelength...

\[ A_\lambda = c \varepsilon_\lambda \]

... is now applied to the whole spectrum:

\[ D = CS^T + R \]
Multivariate analysis: two i-motifs have been detected

The whole dataset was analyzed: D

Calculated concentration profiles: C

Calculated spectra for each species: S
Melting experiments (i)

Melting experiments have been carried out from pH 7 to pH 3

In all cases, multivariate data analysis has been applied

Melting at pH 6.1
Melting experiments (ii)

Results of Van’t Hoff analysis:

<table>
<thead>
<tr>
<th>pH</th>
<th>Tm (°C)</th>
<th>Hyperchro nicity at 295 nm (%)</th>
<th>ΔG° at 20 °C (kcal mol⁻¹)</th>
<th>ΔH° (kcal mol⁻¹)</th>
<th>ΔS° (cal K⁻¹ mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>63</td>
<td>+14</td>
<td>-6.6</td>
<td>-52</td>
<td>-153</td>
</tr>
<tr>
<td>4.5</td>
<td>68</td>
<td>-8</td>
<td>-7.0</td>
<td>-50</td>
<td>-148</td>
</tr>
<tr>
<td>4.7</td>
<td>64</td>
<td>-19</td>
<td>-8.0</td>
<td>-61</td>
<td>-182</td>
</tr>
<tr>
<td>5.6</td>
<td>48</td>
<td>-39</td>
<td>-5.5</td>
<td>-63</td>
<td>-196</td>
</tr>
<tr>
<td>6.1</td>
<td>36</td>
<td>-41</td>
<td>-2.5</td>
<td>-49</td>
<td>-157</td>
</tr>
<tr>
<td>6.8</td>
<td>28</td>
<td>-46</td>
<td>-1.1</td>
<td>-49</td>
<td>-164</td>
</tr>
</tbody>
</table>

Melting profile agrees with the concentration profile for i-motif II

At pH ~ 4.3, hyperchromicity ~ 0 and $T_m$ reaches a maximum
A proposal for the resolved species

<table>
<thead>
<tr>
<th>pH</th>
<th>Cytosine State</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral pH</td>
<td>Deprotonated cytosine involved in Watson-Crick base pairs</td>
<td>Hairpin</td>
</tr>
<tr>
<td>pH ~ 6</td>
<td>Cytosines involved in C+·C base pairs</td>
<td>i-motif I</td>
</tr>
<tr>
<td>pH ~ 4</td>
<td>Free cytosines not involved in the i-motif core are probably protonated</td>
<td>i-motif II</td>
</tr>
</tbody>
</table>
A proposal for the resolved species

A possible structure for the i-motif I formed by the bcl-2c sequence:

5' CCC GCC CCC TTC CTC CCG CGC CCG-3'
A similar sequence only forms just one i-motif

The study of the sequence 5’-CCC GTT CCC TTT TTC CCG TGC CCG -3’ (with any free cytosine) seems to show the formation of an i-motif in the pH range 2 - 7.
Interaction with a G-quadruplex ligand: TmPyP4. pH 6.9

At pH 6.9, TmPyP4 interacts with the Watson-Crick hairpin

A 1:2 (DNA:drug) interaction complex is proposed with log $K_{eq} = 11.7 \pm 0.1$
Interaction with a G-quadruplex ligand: TmPyP4. pH 6.1

At pH 6.1, TmPyP4 interacts with the i-motif I:

A clear decrease of CD intensity: the i-motif structure is lost?

A 1:2 interaction complex is proposed with log $K_{eq} = 12.4 \pm 0.2$

pH 4.2

At pH 4.2, TmPyP4 interacts with the i-motif II:

The structure is not altered. Intercalation?

A 1:1 interaction complex is proposed with log Keq = 6.7 ± 0.6
Conclusions

- The studied sequence forms two i-motif structures in the pH range 2 – 7.
- Stability of the i-motif structures is higher at pH ~ 4.3.
- Interaction with TmPyP4 at pH 7 and pH 6 produces a similar product, where TmPyp4 seems to intercalate into DNA.
- Interaction with TmPyp4 at pH 4 does not produce any change on i-motif structure.
Acknowledgments

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Thank you!!

More info at: www.ub.es/gesq/dna