Convergence?

Stability of RNA hairpins
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RNA Tetraloops – common motifs involved in tertiary contacts, protein binding, folding initiation

Of 256 possible tetraloops, 12 dominate in nature.

UNCG, GNRA, CUYG

~50% of rRNA tetraloops are GNRA loops:
Stability of a GNRA tetraloop
experimental destabilizing effects of point mutations

ΔG=1.3 kcal/mol
ΔG=0.65 kcal/mol
ΔG=0.75 kcal/mol


G

Minor groove

N9

Minor groove

A

Minor groove

N9

Minor groove

I

What is happening?

G → I: removal of NH$_2$ in minor groove

G → A: also loss of N-H at position 1

- G8 → I8 in the stem, loss of one H-bond (1.3 kcal/mol)
- G4 → I4 in the loop, loss of two H-bonds (0.65 kcal/mol)
- G4 → A4 in the loop, loss of three H-bonds (0.75 kcal/mol)
ACAA loop
no specific A4-A7 interactions
FEP simulations

\[
\begin{align*}
\Delta G_{u \rightarrow h}^{G} & \quad \Delta G_{u \rightarrow h}^{l} \\
\downarrow & \\
U^{G} & \rightarrow \quad H^{G} & \rightarrow \quad H^{l} \\
\downarrow & \\
U^{l} & \rightarrow \quad H^{l}
\end{align*}
\]

Models of unfolded state

<table>
<thead>
<tr>
<th></th>
<th>Average $\Delta G_u$ [kcal/mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Guanosine</td>
<td>$73.6 \pm 0.1$</td>
</tr>
<tr>
<td>CGC triplet in A-RNA conformation</td>
<td>$73.9 \pm 0.2$</td>
</tr>
<tr>
<td>CGC triplet from the loop</td>
<td>$74.4 \pm 0.2$</td>
</tr>
<tr>
<td>GCGCA in A-RNA conformation</td>
<td>$73.6 \pm 0.1$</td>
</tr>
</tbody>
</table>

Calculated (PERT) hairpin stabilities

Sensational!

\[ \Delta \Delta G_{\text{calc}} = 1.7 \pm 0.7 \]
\[ \Delta \Delta G_{\text{exp}} = 1.3 \]

\[ \Delta \Delta G_{\text{calc}} = 3.3 \pm 0.7 \]
\[ \Delta \Delta G_{\text{exp}} = 0.65 \]

\[ \Delta \Delta G_{\text{calc}} = 2.9 - 6.2 \]
\[ \Delta \Delta G_{\text{exp}} = 0.75 \]

OK!
Auguste Compte, Philosophie Positive (1830):

“Every attempt to employ mathematical methods in the study of chemical questions must be considered profoundly irrational and contrary to the spirit of chemistry. If mathematical analysis should ever hold a prominent place in chemistry - an aberration which is happily almost impossible - it would occasion a rapid and widespread degeneration of that science.”
(mis?)calculated hairpin stabilities

TABLE 8 Free energy perturbation results from simulations in water sphere [kcal/mol]

<table>
<thead>
<tr>
<th>Mutation</th>
<th>$\Delta G^*$ (forward/backward)</th>
<th>$\Delta G_{\text{AVG}}$</th>
<th>$\Delta \Delta G_{\text{CALC}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfolded $^\dagger$ G4→A4 $^\ddagger$</td>
<td>74.4/−74.2</td>
<td>74.3</td>
<td></td>
</tr>
<tr>
<td>Hairpin G4→A4 $^\S$</td>
<td>79.6/−74.7</td>
<td>77.2</td>
<td>2.9</td>
</tr>
<tr>
<td>Hairpin G4→I4</td>
<td>77.0/−78.0</td>
<td>77.5</td>
<td></td>
</tr>
<tr>
<td>Hairpin I4→A4</td>
<td>4.2/−1.8</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Hairpin G4→A4 via I4</td>
<td>81.2/−79.8</td>
<td>80.5</td>
<td>6.2</td>
</tr>
<tr>
<td>Hairpin I4→G4 $^|$</td>
<td>−74.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hairpin A4→G4 via I4 $^|$</td>
<td>−75.8</td>
<td></td>
<td>4.2</td>
</tr>
</tbody>
</table>

Conformational heterogeneity of the ACAA loop (when imposed on a GCAA structure)
Conclusion

crucial for the rational design of RNA-derived molecules of therapeutic application. Our study suggests that GNRA tetraloops where G is replaced by I or A may have other low-energy conformations distinct from the GNRA fold, which were not reached during FEP simulations. This observation is consistent with the idea that the phylogenetic preference

Note added in proof: After this paper was accepted, the structure of an ACAA tetraloop was determined by NMR (Staple, D. W., S. E. Butcher. 2003. Solution structure of the HIV-1 frameshift inducing stem-loop RNA. *Nucl. Acids. Res.* 31:4326–4331) and found to be of the AGNN-tetraloop type, rather than of the GNRA-type. This confirms our suggestion that
ACAA – sim vs real

GCAA

A7
A6
C5

ACAA

NMR ACAA

A7
A6
C5

ACAA

our ACAA

G4

A7
A6
C5
Conformational Equilibria

Stacking Free Energies from PMF Calculations on Dinucleotides

- Sequence
- Length
- Temperature
- DNA / RNA
- Solvent

![Graph of PMF, Surface Area, and Base Angle vs. R_{N9-N9} [Å]](

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Temperature dependence of ApA stacking from PMF calculations

Figure 4. Van’t Hoff plot for adenylyl-3′,5′-adenosine. The line is
Conformational Equilibria

• Sequence
  Pu-Pu > Pu/Py > Py-Py

• Temperature
  Enthalpy driven
  ΔH = -6 kcal/mol
  ΔS = -13 cal/mol/K

• DNA/RNA
  T better than U
  2’OH - slight favor of stack

  • Primarily nearest neighbor effect
  • High dielectric solvents favor stacking
  • Base mobility decreases stacking