Lecture 7: Base Stacking

Reading: BCT Chpt 2 (pp23-43)

Lecture Review

- In the previous lecture, we reviewed the torsion angles of nucleic acids including some recent literature on the topic. We learned that folding motifs can be identified by their positions on pseudotorsion Ramachandran plots, and that steric exclusion alone tells us much about the predisposition of nucleic acids to certain shapes. We also reviewed the possible conformations about the glycosidic bond and the electronic distributions of nucleic acids. Lastly, the nature and energetics of hydrogen bonding were discussed.

Background

It is generally recognized that there are four principles that guide the conformations of nucleic acids: electrostatics, hydrogen bonding, stacking and shape selectivity. We considered electrostatics and hydrogen bonding, as well as an introduction to shape (in Murthy et al.) in the last lecture.

Base Stacking  A.) Experiments and Trends

- On the basis of what we learned about hydrogen bonding, we might expect that an aqueous solution of G and C would spontaneously arrange to give GC base pairs, and that a solution of A and T would give AT base pairs; i.e.: we might expect edge-to-edge horizontal interactions. However, this is not the case. What is observed is stacking of the bases. Stacking refers to a non-covalent association involving vertical interactions of the bases. This arrangement is similar to coins stacked up in a roll.

- Interestingly, in both the gas phase and aprotic solvents, horizontal interactions with hydrogen bonding are observed. One reason for this may be that there is no thermodynamic penalty to desolvate the hydrogen bond donor and acceptors in the gas phase.

- On the basis of these observations, we conclude that in water stacking may be as important, or more important, energetically than hydrogen bonding.

- Some trends:
  - Purines stack better than pyrimidines
  - Aromatic amino acids (trp, tyr, phe) can stack with the nucleobases. This contributes to amino acid recognition.
Base Stacking  B.) Thermodynamics

- We will look at base stacking three different ways: stacking of monomers, dangling ends, and model compounds—the latter will be handled with the L4 in-class discussion.

### i. Monomers

#### Table 2.2

<table>
<thead>
<tr>
<th>Nucleoside</th>
<th>$K(25^\circ)$ (M$^{-1}$)</th>
<th>$\Delta G^\circ(25^\circ)$ (kcal mol$^{-1}$)</th>
<th>$\Delta H^\circ$ (kcal mol$^{-1}$)</th>
<th>$\Delta S^\circ$ (eu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uridine</td>
<td>0.6</td>
<td>+0.3</td>
<td>−2.7</td>
<td>−10</td>
</tr>
<tr>
<td>Thymidine</td>
<td>0.9</td>
<td>+0.1</td>
<td>−2.4</td>
<td>−9</td>
</tr>
<tr>
<td>Cytidine</td>
<td>0.9</td>
<td>+0.1</td>
<td>−2.8</td>
<td>−10</td>
</tr>
<tr>
<td>Deoxyctydine</td>
<td>0.9</td>
<td>+0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenosine</td>
<td>5</td>
<td>−1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deoxyadenosine</td>
<td>5−8</td>
<td>−1 to −1.2</td>
<td>−6.5</td>
<td>−18</td>
</tr>
</tbody>
</table>

Data are from Ts' o, (1974). Guanosine could not be studied because of its low solubility.

- Equations needed here are:
  
  \[
  \Delta G^\circ = -RT \ln K \quad \text{eq 4.1}
  \]
  
  \[
  \frac{\partial \ln K}{\partial \frac{1}{T}} = -\frac{\Delta H^\circ}{R} \quad \text{(this is the van’t Hoff equation)} \quad \text{eq 4.2}
  \]
  
  \[
  \Delta G^\circ = \Delta H^\circ - T \Delta S^\circ \quad \text{eq 4.3}
  \]

- $\Delta G^\circ$ is close to zero, which means that $K$ is close to 1 (eq 4.1).

- Note that $\Delta H^\circ$ for stacking is favorable (i.e.: the $\Delta H^\circ$ part of $\Delta G^\circ$ is negative) and $\Delta S^\circ$ is unfavorable (i.e.: the $-T\Delta S^\circ$ part of $\Delta G^\circ$ is positive). The unfavorable-favorable pairing of $\Delta S^\circ$ and $\Delta H^\circ$, respectively, is an example of entropy-enthalpy compensation.

- Classically, the hydrophobic effect involves ‘squeezing’ out of water molecules from the monomers, which is entropically favorable. Thus, the negative sign on $\Delta S^\circ$ for nucleic acid stacking suggests that the hydrophobic effect is not important in base stacking.

- The negative sign on $\Delta H^\circ$ suggests that the electronic nature of the interactions is important.
ii. Dangling Ends

- In the above figure, note that single-stranded polymers form a stacked structure (labeled as ‘Intrastrand Stacking’); they are not just random coils. We will examine detailed models for the equilibria for this later in the semester.

- Note also that dangling ends (labeled as ‘Interstrand Stacking’) contribute quite significantly to helix stability (see Math Box below for quantitation hints). In some instances a dangling end can contribute as much to helix stability as a Watson-Crick base pair can!

### Math Box:
Each increment of 1.4 kcal/mol in $\Delta G^\circ_{37}$ is worth $\approx 10$-fold in $K_{eq}$
(i.e. use eq 4.1: $\Delta G^\circ_{37} = -(0.001987 \text{kcal K}^{-1} \text{mol}^{-1})(273.15+37) \text{K} \text{ln}10 = -1.4 \text{ kcal mol}^{-1}$.)

I keep the following rule of thumb in my head and use it when I’m unable (or too lazy) to grab my calculator.

<table>
<thead>
<tr>
<th>$\Delta G^\circ_{37} (\text{kcal mol}^{-1})$</th>
<th>$K_{eq}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1.5</td>
<td>10</td>
</tr>
<tr>
<td>-3.0</td>
<td>100</td>
</tr>
<tr>
<td>-4.5</td>
<td>1000</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

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2. Nucleic Acid chemists most often use kcal/mol. However, you will also find kJ/mol. The conversion factor is 4.184 J/cal; so each 1.4 kcal/mol is 5.9 kJ/mol.
Base Stacking  

C.) Structures

- For RNA, a 3'-dangling end stacks better than a 5'-dangling end. For DNA, it is the other way around: a 5'-dangling end stacks better than a 3'-dangling end. ³ See this section for structural bases for these effects.

- A common theme to biophysical chemistry is the interplay of structure and function. There must be some structural basis for preferred stacking of a 3'-dangling end in RNA.

Structure 1

- The following study shows that a 3'-dangling end can stack on the opposite strand and stabilize the duplex (see panel b). The 5'-dangling end does not do this.

There is a strong correlation between the thermodynamics of stacking in model duplex systems (from the simple dangling end studies above) and actual stacking of dangling ends in larger RNA structures.

For the 36 unpaired bases having sequences with $\Delta G_{37}^{\circ \text{stack}}$ more favorable than $-0.7$ kcal/mol, 83% are found to be stacked on the adjacent base pair in the larger RNA.

For the 56 unpaired bases having sequences with $\Delta G_{37}^{\circ \text{stack}}$ less favorable than $-0.4$ kcal/mol, only 34% are found to be stacked on the adjacent base pair in the larger RNA.

The following Figure shows this structurally. (Panel a) A stable 3′-dangling end stack (CU/G), stacks on its own strand and the opposite strand and stabilizes the duplex. Here the favorable overlap appears to be primarily of the carbonyl of U7 with the amino of C6, which have complementary dipoles. (Panel b) A low stability 3′-dangling end stack (UU/A) does not stack. Poor 3′-end stackers are good places for turns in a structure.

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