Thermodynamics of DNA Duplex Formation

Objective
Determine $\Delta H^\circ$, $\Delta S^\circ$, and $\Delta G^\circ$ for the transition from the single strand state to the double helix or duplex state of a small oligomer from melting curves.

Introduction
In this lab you will measure thermodynamic properties of a short DNA duplex by melting the ordered native structure (duplex or double helix) into the disordered, denatured state (single strands) while monitoring the transition using ultraviolet (UV) spectrophotometry.

As the ordered regions of stacked base pairs in the DNA duplex are disrupted, the UV absorbance increases. This difference in absorbance between the duplex and single strand states is due to an effect called hypochromicity. Hypochromicity, which simply means “less color”, is the result of nearest neighbor base pair interactions. When the DNA is in the duplex state, interactions between base pairs decrease the UV absorbance relative to single strands. When the DNA is in the single strand state the interactions are much weaker, due to the decreased proximity, and the UV absorbance is higher than the duplex state. The profile of UV absorbance versus temperature is called a melting curve; the midpoint of the transition is defined as the melting temperature, $T_m$. The dependence of strand concentration on $T_m$ of a melting transition can be analyzed to yield quantitative thermodynamic data including $\Delta H^\circ$, $\Delta S^\circ$, $\Delta G^\circ$ for the transition from duplex to single strand DNA.

Thermodynamic analyses of this type are done extensively in biochemistry research labs, particularly those involved in nucleic acid structure determination. In addition to providing important information about the conformational properties of either DNA or RNA sequences (mismatched base pairs and loops have distinct effects on melting properties), thermodynamic data for DNA are also important for several basic biochemical applications. For example, information about $T_m$ can be used to determine the minimum length of a oligonucleotide probe needed to form a stable double helix with a target gene at a particular temperature.

This handout is adapted from the supporting information in this article: Howard, K. P. Thermodynamics of DNA Duplex Formation: A Biophysical Chemistry Laboratory Experiment. J. Chem. Educ. 2000, 77, 1469
**Procedure**

You will receive data collected from melting a duplex formed by two complementary synthetic DNA oligomers.

\[(5')\text{CAAAAAAAG} \text{ and } (5')\text{CTTTTTTTG}\]

Five separate samples with different concentrations (indicated on cuvettes) were prepared in buffer (1 M NaCl, 10 mM sodium phosphate pH 7, 0.1 mM disodium EDTA). The buffer was degassed by bubbling nitrogen through it before the samples were made. Oxygen dissolved in the sample will form bubbles at higher temperatures, which will scatter light and affect the absorbance measurements. The samples (2 mL each) were placed in sealed cuvettes. Five additional cuvettes filled with buffer were used as reference cells in the double-beam spectrophotometer.

During the melting experiment, the absorbance was monitored at 260 nm as temperature was increased at 2 °C/min. The spectrophotometer parameters used are shown in Table I.

<table>
<thead>
<tr>
<th>Temperature Parameter</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>10</td>
</tr>
<tr>
<td>Sampling interval</td>
<td>2</td>
</tr>
<tr>
<td>Final</td>
<td>70</td>
</tr>
<tr>
<td>Post-Experiment</td>
<td>10</td>
</tr>
</tbody>
</table>

To record the most accurate data in a research laboratory, melting curves of this type would generally be done slowly (over several hours) at small temperature increments to ensure complete temperature equilibration at each point. This experiment has been designed to fit into a single lab period by minimizing the amount of time necessary to equilibrate at each temperature by the choice of particular DNA duplex and the use of small sample volumes. Nonetheless, you should be aware that incomplete temperature equilibration could be a source of error in these measurements.

**Calculations**

The data is provided to you in an Excel file with five pairs of columns. Each pair of columns has a temperature (°C) column and an absorbance column. The five data sets are for 1, 2, 4, 8, and 16 μM samples of double-stranded DNA, respectively.
Your first step will be to make a single graph of normalized absorbance as a function of temperature that contains the five melting curves. Melting curves of DNA are commonly described using standard helix-to-coil transition theory. In our case the "helix" is duplex DNA and the "coil" is the disordered single DNA strands. The transition from helix to coil is monitored in our experiment as a function of temperature by UV absorbance. This can be done because the percentage of hyperchromicity (increase in absorbance as the duplex is melted) varies linearly with the number of unstacked bases. Our melting curve relates the absorbance to the fraction of dissociated bases \( f \) as the temperature is increased. The \( T_m \) is the temperature where \( f=0.5 \).

The steep part of the melting curves reflects the double strand \((AB)\) to single strand \((A+B)\) equilibrium.

\[
AB \rightleftharpoons A + B \tag{1}
\]

The treatment we used assumes a two-state (all-or-none) model. In a two-state model, \( f \) is the fraction of fully dissociated strands since there are no partially base-paired intermediates in the melting process. The two-state model has been shown to be a very good approximation for short (<12 base pairs) DNA duplexes. Using this model we must normalize the absorbance data so that the values range from 0 to 1 (we will call these values relative absorbance). A relative absorbance of 0 occurs when all of the bases are paired (all in the duplex state) and a relative absorbance of 1 occurs when all of the bases are un-paired (all in the single strand state). To normalize your data, subtract the lowest value for each curve from all the values in that curve. Then, divide each value by the maximum of the subtracted values. The normalized data can then be fit with a sigmoidal curve.

\[
f(T) = \frac{1}{1 + e^{D(T_m-T)}} \tag{2}\]

In this equation, \( T_m \) is the melting temperature for the DNA, and \( D \) is an empirical fitting parameter. You will obtain thermodynamic data from the concentration dependence of \( T_m \) for each of your curves.

Next make a van’t Hoff plot of \((1/T_m)\) versus \( \ln(C_t) \) where \( C_t \) is the sum of the molar concentrations of each single strand. Then, use equation 3 to calculate \( \Delta H^\circ \) and \( \Delta S^\circ \).

\[
\frac{1}{T_m} = -\frac{R}{\Delta H^\circ} \ln C_t + \frac{(\Delta S^\circ + R \ln 4)}{\Delta H^\circ} \tag{3}\]
Finally, calculate $\Delta G^\circ$ at 25°C. Note that some sources use a slightly different form than in equation 3, the difference is that here we take the forward direction to be consistent with what is written in equation 1.

**Things to include on your ONE-PAGE report.**
- Normalized melting curves on one graph.
- van’ Hoff plot.
- $\Delta H^\circ$ and $\Delta S^\circ$ for helix formation.
- $\Delta G^\circ$ at 25°C for helix formation.
- Literature values for $\Delta H^\circ$, $\Delta S^\circ$, and $\Delta G^\circ$.
- All appropriate errors.

**References**
Marky et al. (1987) Biopolymers 26, pp 1601-1620