Thermodynamics of DNA Duplex Formation

A Biophysical Chemistry Laboratory Experiment

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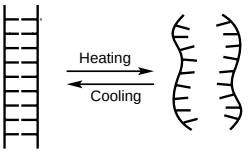
The goal of this experiment is to measure thermodynamic properties of a short DNA duplex by melting the ordered native structure (duplex) into the disordered, denatured state (single strands) while monitoring the transition using ultraviolet (UV) spectrophotometry.



As the ordered regions of stacked base pairs in the DNA duplex are disrupted, the UV absorbance increases. The profile of UV absorbance versus temperature is called a melting curve and the midpoint of the transition is defined as the melting temperature, T_m. The dependence of T_m on strand concentration can be analyzed to yield quantitative thermodynamic data ($\Delta H°$, $\Delta S°$, $\Delta G°$). Thermodynamic analyses of this type are routine in many biophysical research labs, particularly those involved in nucleic acid structure determination (*1, 2*). Thermodynamic information derived from DNA melts has helped in understanding the sequence dependence and polymorphism in the secondary structure of nucleic acids (mismatched base pairs and loops have distinct effects on melting properties) (*3*). In addition to providing important information about conformation, knowledge of thermodynamic data for DNA is essential for several basic biochemical applications. For example, one must know the T_m to determine the minimum length of a probe oligonucleotide needed to form a stable double helix with a target gene at a particular temperature.

The experiment described here was devised for a newly developed course in Biophysical Chemistry, which is part of the Physical Chemistry requirement for our Biochemistry majors. The goal of this course is to demonstrate the important role physical chemistry plays in understanding the structures and properties of biological macromolecules. A challenge for us has been to incorporate real biophysical experiments into the laboratory component of the course despite the lack of such experiments in traditional physical chemistry textbooks. A few biophysical laboratory experiments have been published recently (*4, 5*), but there is clearly a need for more.

Methods

Equipment

Any standard commercial UV spectrophotometer with temperature control can be equipped to measure melting curves. We used a Cary 1 UV–vis spectrophotometer equipped with a multicell block and transport apparatus. This enabled us to complete the melts of four different samples at the same time and have each student collect his or her own full set of data to analyze. Without a multicell block, arrangements can be made to have different students collect different concentration points and have the class share data. A set of quartz cuvettes that can be sealed to prevent evaporation during heating is also required. Plotting and analysis of the melting curves can be done using a graphing software program like Kaleidagraph (*6*).

DNA Melt

The DNA samples we used were two complementary synthetic DNA oligomers, dCA_7G and dCT_7G. Four separate samples with different concentrations (each single strand between 10 and 75 μM) were prepared in degassed buffer (1 M NaCl, 10 mM sodium phosphate pH 7, 0.1 mM disodium EDTA).[1] The samples (0.4 mL each) were then placed in 1-mm pathlength quartz cuvettes and sealed with Teflon stoppers.

The denaturation of DNA duplex was monitored by measuring the change in absorbance at 260 nm over the temperature range 10 to 70 °C. The melting-experiment data were collected on a spectrophotometer equipped with a multicell block and transport apparatus, which allowed the simultaneous measurement of the four samples. To record the most accurate data in a research laboratory, DNA melts of this type are often done slowly (over several hours) at small temperature increments, to ensure complete temperature equilibration at each point (*2*). However, this single-lab-period experiment was designed to minimize the amount of time necessary to equilibrate at each temperature by the choice of a short DNA duplex and the use of small sample volumes. After the melting experiment, the samples were cooled to the starting temperature and equilibrated for at least 10 minutes, and the absorbance at 260 nm was recorded for each of the four samples. This value was compared to the absorbance at 260 nm at the start of the experiment to see if there was a rise in absorbance, which could indicate evaporation of the buffer or hydrolysis of the sample.

Data Analysis

Melting curves of DNA are commonly described using standard helix-to-coil transition theory. In this 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 approximately linearly with the number of unstacked bases (*7*). Thus the melting curve relates the absorbance to a profile of fraction of bases paired (f) versus temperature. The T_m is the temperature at which $f = 0.5$.

The steep part of the melt curves reflects the double-strand (A_2) to single-strand (A) equilibrium

$$A_2 \rightleftharpoons 2A$$

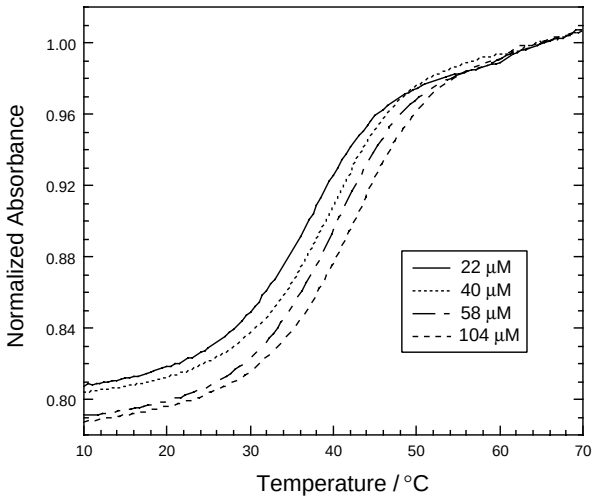


Figure 1. Melting curves showing absorbance versus temperature for four total concentrations (C_t) of dCA_7G and dCT_7G in 1 M NaCl, 10 mM sodium phosphate pH 7.0, 0.1 mM sodium EDTA. All the curves are normalized to an absorbance of 1 at 65 °C.

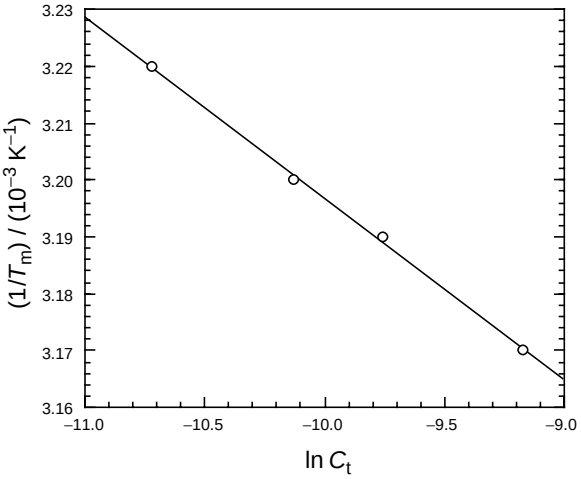


Figure 2. van't Hoff plot of $1/T_m$ vs ln C_t where T_m is the melting temperature and C_t is the total single-strand concentration. The line shown ($y = 2.88 - 0.032x$) is the best least-squares fit to the data. $R^2 = .999$.

The treatment we used assumes a two-state (all-or-none) model. (In a two-state model, f is the fraction of fully base-paired 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 (*2*).

Data files for each of the four melts were transferred from the spectrophotometer to a data disk and then opened in a graphing software program. Thermodynamic data for the formation of a DNA duplex can be determined from the concentration dependence of the T_m. The T_m of each absorbance-versus-temperature curve can be determined by taking the first derivative to find the inflection point. (Taking the first derivative of a curve is straightforward in a many graphing software programs.) The maximum of the first derivative curve is a good approximation of the T_m.

Next a van't Hoff plot of $\ln(C_t)$ versus $(1/T_m)$ was made, where C_t is the sum of the molar concentrations of each single strand and T_m is expressed in K. Using the following relationship (*2, 8*)

$$\frac{1}{T_m} = \frac{R}{\Delta H^\circ} \ln C_t + \frac{(\Delta S^\circ - R \ln 4)}{\Delta H^\circ} \qquad (1)$$

both ΔH° and ΔS° were calculated. Finally, ΔG° was calculated at 25 °C.

Results and Discussion

Figure 1 shows melting curves for four concentrations of DNA. As clearly shown, the curves shift to higher temperatures as the concentration is increased. As part of the lab write-up students are asked to explain using simple thermodynamic relationships why the concentration should affect the observed T_m (*8*). Figure 2 shows a van't Hoff plot using the T_m values determined from the melting curves. The slope and intercept of the line in Figure 2 were used to calculate ΔS° and ΔH° using eq 1. The values calculated from the data shown here are $\Delta H^\circ = -62.1$ kcal/mol and $\Delta S^\circ = -176$ cal/deg/mol. These values are typical of the results obtained by students in the Biophysical Chemistry course taught at Swarthmore College in the past two years. The values are in good agreement with data in the literature: $\Delta H^\circ = -68.0$ kcal/mol and $\Delta S^\circ = -196$ cal/deg/mol (*3*). The literature also contains data on several closely related DNA duplexes, which students can compare with their own results (*3*).

Conclusions

This laboratory presents the opportunity for students to do careful physical measurements on a biomacromolecule. The experiment provides reliable data, which students can analyze using basic thermodynamic principles and from which they can calculate parameters that provide insight into DNA structure. In addition to practice with laboratory technique and data analysis, this lab touches upon several key concepts that can be discussed in the lecture component of a biophysical chemistry class and reinforced with questions in the student lab handout. Important concepts involved with this lab include cooperativity of structural transitions, Beer's law, and the hypochromic effect arising from electronic interaction between neighboring stacked bases.

Acknowledgments

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Supplemental Material

A student handout and detailed set of instructor notes for setting up this experiment are available in this issue of *JCE Online*.

Note

1. This experiment requires two complementary DNA oligomers (dCA_7G) and (dCT_7G). DNA can be ordered by custom synthesis from any of a large number of private companies or university-based nucleic acid facilities. (For $50 total, we were able to order a one-micromole synthesis for each of the DNA strands, which provided between 1.5 and 3.0 mg of each strand—more than enough DNA for two years' worth of student experiments.) The

materials for the buffer were purchased from Sigma-Aldrich (Milwaukee, WI): NaCl (CAS# 7647-14-5), sodium phosphate monobasic (CAS# 7558-80-7), sodium phosphate dibasic (CAS# 7558-79-4), and disodium EDTA (CAS# 6381-92-6).

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