

Analysis of the Melting Temperature and Thermodynamic Parameters of a Nucleic Acid using a UV-Visible Spectrophotometer

Introduction

In recent years the use of oligonucleotide based therapeutics derived from nucleic acids such as DNA or RNA, has increased significantly. They are considered to be next-generation drugs due to two important factors: firstly, they act not only on proteins that have traditionally been targeted by conventional small-molecule or antibody base drugs, but also on functional RNA such as mRNA and miRNA, and secondly they can be manufactured by chemical synthesis, which means that they can be easily mass produced. Oligonucleotide therapeutics are mainly based on artificial oligonucleotides that are chemically modified DNA or RNA, which improves their structural stability in the human body and affinity to their target molecules¹). The evaluation of their interaction characteristics with target molecules and use as drugs, optimization of their number of the bases and sequence are critical. Thus, the importance of the examination and evaluation methods for the optimization of these properties has been well defined. The importance of the thermodynamic properties has been highlighted, and therefore an efficient method to analyze these characteristics is required.

This application note reports the evaluation of the melting temperature and thermodynamic parameters of a nucleic acid sample. The absorbance at 260 nm was measured for different sample concentrations while ramping the temperature²) using a V-700 Series UV-visible spectrophotometer with PAC-743 Automatic 6/8-position Peltier cell changer, which can measure up to eight samples simultaneously.

Keywords

Oligonucleotide therapeutic, oligonucleotide, nucleic acid, thermodynamic parameter, enthalpy, entropy, PCR, Southern blot, intracellular environment, molecular crowding

Melting Temperature

When double-stranded DNA (dsDNA) is heated, interaction forces that contribute to the formation of double strand such as hydrogen bonding between the bases pairs is weakened, resulting in the separation of dsDNA into two single-stranded DNA (ssDNA). This dissociation is described as DNA melting (Fig. 1), and the temperature at which a half of the dsDNA dissociates into ssDNA is defined as the melting temperature (T_m).

Nucleobases that compose DNA, RNA and artificial oligonucleotides almost all have an absorbance in the UV region, and usually with a peak maxima at around 260 nm. For example, in dsDNA, the bases are arranged with a stacking interaction, which makes the absorbance of the whole dsDNA smaller than the sum of the absorbance of each base. However, when dsDNA dissociates into ssDNA, the bases are no longer bonded resulting in a larger absorbance than for dsDNA. Using this phenomenon, the T_m can be calculated by measuring the absorbance in the UV region while ramping the temperature.

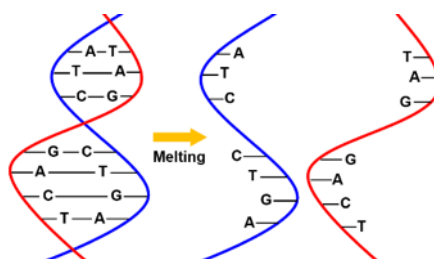


Figure 1. A schematic diagram of DNA melting

Thermodynamic Parameters

Free energy (ΔG°) is expressed by the following equation, and a reaction or complex formation happens spontaneously when ΔG° is negative (Fig. 2).

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (1)$$

It is well known that there is a correlation between enthalpy change (ΔH°) and entropy change (ΔS°) in most chemical and biological interactions. This correlation is described as the enthalpy-entropy compensation: when ΔH° is favorable ($\Delta H^\circ < 0$), ΔS° tends to be unfavorable, on the other hand, when ΔH° is unfavorable ($\Delta H^\circ > 0$), ΔS° tends to be favorable. Temperature only affects the term of entropy change. In forming dsDNA, the formation of hydrogen bonds and some other interactions result in a favorable $\Delta H^\circ (< 0)$, and a decrease in molecular disorder results in an unfavorable $\Delta S^\circ (< 0)$, thus $-T\Delta S^\circ$ is positive. On the other hand, in the dissociation of dsDNA, the rise in temperature causes $-T\Delta S^\circ$ to be greater than ΔH° resulting in a positive ΔG° , which as a result causes the dissociation. As ΔH° becomes larger more duplex is formed, the less the molecular disorder gets the more unfavorable ΔS° becomes. This is the reason why the correlation is described as 'compensation'.

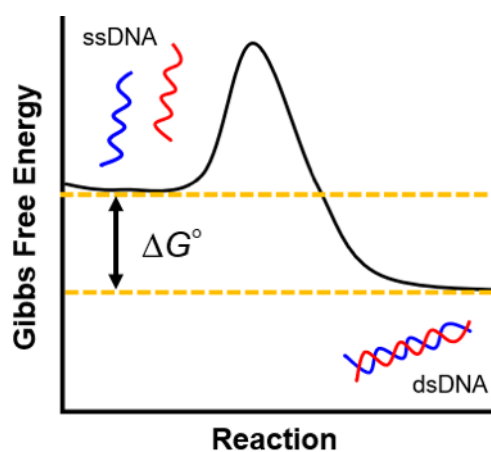


Figure 2. Free energy change of double helix formation of DNA

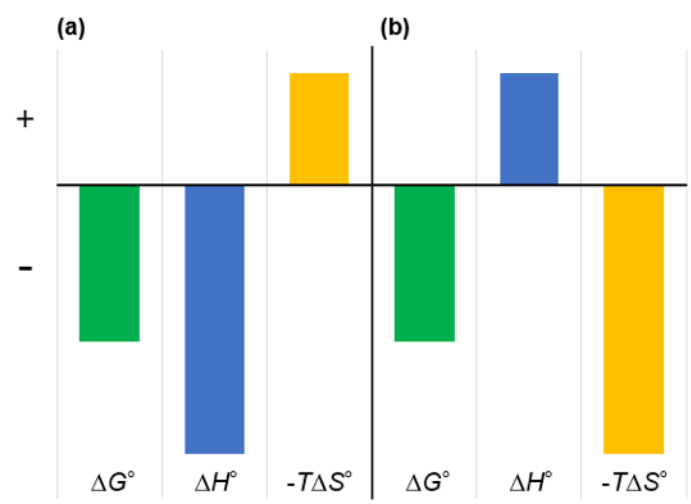


Figure 3. A schematic diagram of thermodynamic profiles of molecular interaction often found in biology. (a) Enthalpy-driven interaction in which hydrogen bonding is the driving force. (b) Entropy-driven interaction in which hydrophobic effect is the driving force.

In a molecular interaction and complex formation, it is considered to be enthalpy-driven when the effect of negative ΔH° on ΔG° is larger than that of negative ΔS° , on the other hand, when the effect of positive ΔS° on ΔG° overcomes that of positive ΔH° , it is considered to be entropy-driven (Fig. 3). In biomolecule interactions occurring in water, hydrogen bonding is often estimated to be the driving force of enthalpy-driven interactions, and the hydrophobic effect is estimated to be the driving force of entropy-driven interactions^{3),4)}. Therefore, the evaluation of ΔH° and ΔS° enables the estimation of the driving force and tendency of the interaction. As a result, it can be used in the estimation of complex formations and the molecular design of more stable complexes.

Experimental

The melting curves of 4 $\mu\text{mol/L}$, 8 $\mu\text{mol/L}$, 20 $\mu\text{mol/L}$ and 40 $\mu\text{mol/L}$ * of the nucleic acid (5'-TGCAAGACTATAAGATTC-3') solution were measured. The measurements were conducted using a V-730 UV-visible Spectrophotometer with PAC-743 Automatic 6/8-Position Peltier Cell Changer. The samples were prepared in 8-position micro-cell. For the measurement and analysis, VWTP-959 Temperature Control Measurement/DNA Melting Analysis was used.

*These are the concentrations of ssDNA after dissociation of dsDNA.

Features of PAC-743 Automatic 6/8-Position Peltier Thermostatted Cell Changer

- Up to 8 samples are measured in a single run
- Very small amount of sample
e.g. 100 μL for 10 mm pathlength cell, 10 μL for 1 mm pathlength cell, etc.
- Temperature is accurately monitored during measurement by probe sensor
- Preventing sample evaporation by sealing caps



Figure 4. PAC-743 and 8-position micro-cell

Features of DNA Melting Analysis Program

- Calculation of T_m , ΔH° , ΔS° , and GC-content
- High-accurate calculation of thermodynamic parameters by $1/T_m$ vs. $\ln(C_t/4)$ plot**
- ** C_t stands for the molar concentration of ssDNA
- Analysis of both dsDNA composed of two sequence-identical strands and the one composed of two sequence-different strands
- Analysis of multiple data conducted at once

Melting curves of the nucleic acid were measured under the conditions shown below. The T_m of the sample was calculated from these curves, and ΔH° and ΔS° were calculated by $1/T_m$ vs. $\ln(C_t/4)$ plot.

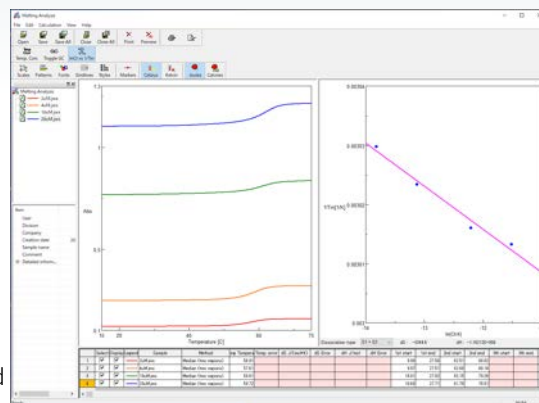


Figure 5. DNA Melting Analysis program

Measurement Conditions

| | | | |
|------------|--------|---------------|-------------|
| Wavelength | 260 nm | Response | 0.96 sec |
| Pathlength | 10 mm | Sample Volume | 100 μ L |

Results

The melting curves for the different concentrations of nucleic acid are shown in Figure 6. The T_m of each solution is shown in Table 1, and $1/T_m$ vs. $\ln(C_t/4)$ plot is shown in Figure 7. ΔH° and ΔS° are calculated from the approximate line of the plot; -578.0 kJ/mol and -1.635 kJ/(mol·K), respectively.

| C_t [mol/L] | T_m [°C] |
|--------------------|------------|
| $4 \cdot 10^{-6}$ | 57.2 |
| $8 \cdot 10^{-6}$ | 58.1 |
| $20 \cdot 10^{-6}$ | 59.8 |
| $40 \cdot 10^{-6}$ | 60.8 |

Table 1. Concentration and melting temperature of nucleic acid solutions

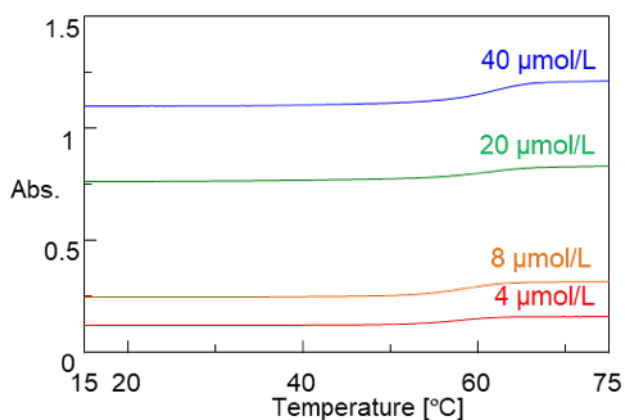


Figure 6. Melting curves

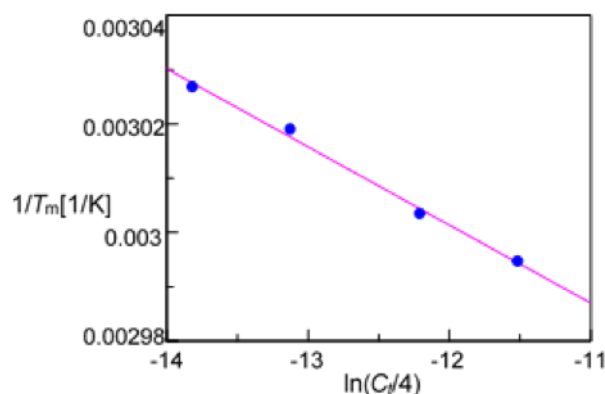


Figure 7. $1/T_m$ vs. $\ln(C_t/4)$ plot

Conclusion

The results show that the enthalpy change greatly overcomes the entropy change in dsDNA formation of the nucleic acid solution. This indicates that dsDNA formation of the sample is enthalpy-driven, which suggests that the driving force of the formation is mainly hydrogen bonding and stacking interaction. This observation agrees with the mechanism of dsDNA formation discovered by Watson and Crick.

This application note shows that the PAC-743 provides simultaneous measurements of multiple samples, while accurately monitoring the temperature. Also, evaluation of thermal stability of nucleic acid and stability of complex can be done by calculating T_m , ΔH° and ΔS° using the DNA Melting Analysis program. Needless to say, the evaluation of T_m is also important in the use of PCR (polymerase chain reaction) and Southern blot, which are well-used methods to duplicate or detect DNAs. Moreover, the evaluation of T_m and thermal parameters is key for the estimation of complex formation under a molecular-crowding environment, a pseudo environment of intracellular environment that has also been gathering attention.

We believe that the combination of PAC-743 and DNA Melting Analysis program is suitable for the efficient acquisition of useful information for the development of oligonucleotide therapeutics and for research methods based on the nature of DNA and double-helix formation.

References

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