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Application News

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Evaluating Thermal Stability (Tm) Analysis and Thermodynamic Properties of Nucleic Acid Drugs

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User Benefits

- Determine the temperature at which 50 % of double-stranded DNA separates into single-stranded DNA (Temperature of Melting [Tm]) for nucleic acid drugs.
- Use Tm to determine changes in Gibbs free energy, an indicator of drug activity.
- Derive thermodynamic properties, such as entropy change and enthalpy change.

■ Introduction

Until around 20 years ago, the vast majority of drugs in the pharmaceutical industry were relatively cheap low-molecular-weight drugs. By 2021, antibody drugs and other biopharmaceuticals that have relatively high molecular weights had grown to account for more than 30 % of all sales. Now, recent improvements in drug discovery technology and greater knowledge of the life sciences are expected to accelerate the development and marketing of nucleic acid drugs that are as safe and effective as biopharmaceuticals and that can treat diseases that biopharmaceuticals cannot. Fig. 1 shows these different drugs and their respective merits.

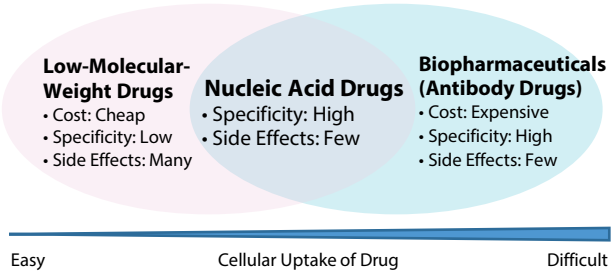

Fig. 1 Types of Drugs and their Merits

■ What are Nucleic Acid Drugs?

Nucleic acid drugs have a base structure that is composed of nucleotides (nucleic acids), the structural components of RNA and DNA. Nucleic acid drugs exert their pharmacological effect by binding specifically and suppressing the activity of mRNA that could not be targeted with conventional low-molecular-weight drugs and biopharmaceuticals. By binding to a target protein, nucleic acid drugs inhibit or activate its function.

Recent advances in techniques for chemically modifying nucleic acids have also led to the development of more effective nucleic acid drugs by reducing degradation by nucleases (enzymes), improving binding to target sequences, and improving the efficiency of cellular uptakes.[1)]

■ Thermal Stability of Nucleic Acid Drugs

The Tm is an indicator of the thermal stability of nucleic acid drugs and is the temperature at which 50 % of double-stranded DNA separates into single-stranded DNA. A higher Tm indicates greater thermal stability.

The thermal stability of nucleic acid drugs can be analyzed by combining a Shimadzu UV-Vis spectrophotometer with the TMSPC-8 Tm analysis system. Please also refer to Shimadzu Application News No. A618 for additional information.

■ Activity Indicators for Nucleic Acid Drugs

The above-mentioned Tm can be used to evaluate indicators of pharmaceutical activity for nucleic acid drugs. One indicator used to verify the activity of nucleic acid drugs is Gibbs free energy. Gibbs free energy represents the amount of non-expansion work and is used to express the maximum amount of work obtained during binding. When the change in Gibbs free energy is negative, the bigger the negative number is the more strongly a given protein binds to other molecules.

Changes in Gibbs free energy are defined thermodynamically and statistically by equations (1) and (2) below, where K is the equilibrium constant and ΔG° is the change in Gibbs free energy under standard conditions (1 atm, 25 °C):

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

$$\Delta G = \Delta G^\circ + RT \ln K \quad (2)$$

When considering the equilibrium constant of nucleic acids, chains A and B can be described as an equilibrium reaction between two molecules that associate in the ratio of 1:1 in equation (3) below:

$$A + B \leftrightarrow AB \quad (3)$$

When the mole fraction of two-chain nucleic acids is α and the total nucleic acid concentration is C, the concentration of A and B when fully dissociated ([A] and [B]) is C/2, hence the equilibrium constant can be expressed as:

$$K = \frac{2\alpha}{C(1-\alpha)^2} \quad (4)$$

In a state of equilibrium when 50 % of nucleic acids separated,

$$\alpha = \frac{1}{2},\ \Delta G = 0 \quad (5)$$

Substituting equations (4) and (5) into equation (2) gives:

$$\Delta G^\circ = -RT_m \ln \frac{4}{C} \quad (6)$$

Knowing not just ΔG°, which indicates the strength of binding, but also which function has a dominant effect on ΔG° will enable more effective drug development.

Substituting equation (6) into equation (1) gives:

$$\frac{1}{T_m} = \frac{R}{\Delta H^\circ} \ln \frac{C}{4} + \frac{\Delta S^\circ}{\Delta H^\circ} \quad (7)$$

When measurements are plotted as 1/Tm and ln(C/4), the intercept and slope of the resulting graph can be used to calculate the enthalpy change ΔH° and the entropy change ΔS° under standard conditions and determine which physical quantity has a dominant effect.

■ Calculation of Tm and Thermodynamic Indicators

The nucleic acid M13-25mer was dissolved in buffer (5 M NaCl, 66.7 mM phosphate buffer solution) to prepare sample solutions of 2 to 60 µM.

An 8-cell multi-micro cell holder was used that is available in two optical path lengths (10 mm and 1 mm), offering a choice between the optical path lengths based on sample absorbance (concentration). The 10 mm optical path length cell requires a minimum sample volume of 100 µL, whereas the 1 mm optical path length cell requires as little as 10 µL, making it particularly useful in the early stages of drug discoveries when only small sample volumes are available. In this study, the lowest concentration sample solutions (2 and 5 µM) were analyzed using 10 mm optical path length cells, and the remaining sample solutions (9 to 60 µM) were analyzed using 1 mm optical path length cells. The analysis conditions are shown in Table 1.

Table 1 Analysis Conditions

Equipment:	UV-2600i TMSPC-8 8-cell multi-micro cell holder Optical path lengths: 10 mm, 1 mm
Measured Wavelength:	260 nm
Measured Wavelength (for Calibration):	320 nm
Slit Width:	5.0 nm
Temperature Range:	15 to 90 °C
Temperature Interval:	1 °C
Heating Rate:	1 °C/min

Fig. 2 shows the change in absorbance versus temperature at concentrations analyzed using a 1 mm optical path length cell.

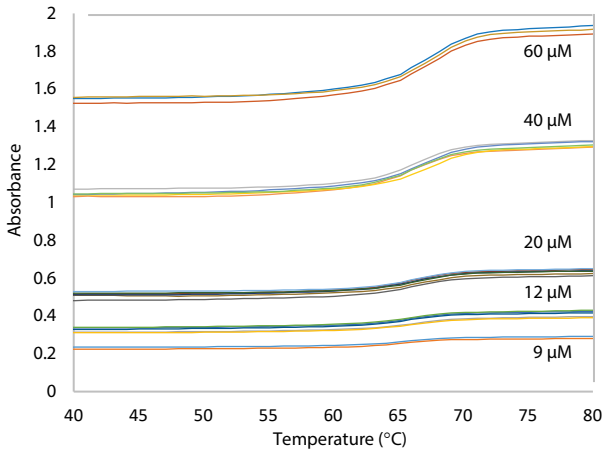

Fig. 2 Melting Curves for Sample Solutions of 9 to 60 µM

Based on this chart, the TMSPC-8 Tm analysis system was used to calculate the Tm at each concentration (Table 2). Next, the inverse of the Tm at each concentration (1/Tm) was plotted against ln(C/4) as shown in Fig. 3. The intercept and slope of Fig. 3 were then used to calculate ΔG°, ΔH°, and ΔS° shown in Table 3.

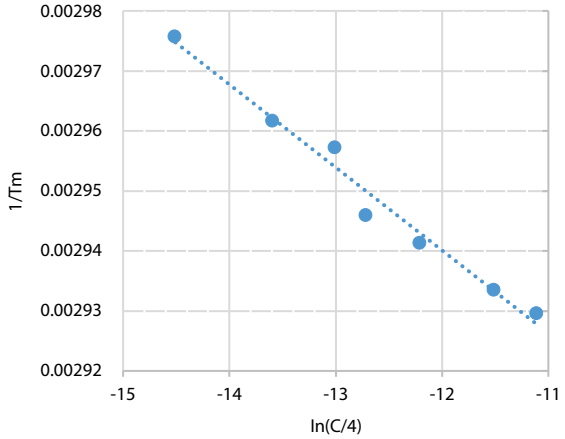

Fig. 3 1/Tm Plotted Against ln(C/4)

Table 2 Tm at Each Concentration

Concentration C (µM)	Tm (°C)
2	62.9
5	64.5
9	65.0
12	66.3
20	66.8
40	67.7
60	68.2

Table 3 Calculated ΔG°, ΔH°, and ΔS°

Factor	Value
ΔG°	-107 kJ/mol
ΔH°	-622 kJ/mol
ΔS°	-1792 J/(mol•K)

The above findings show M13-25mer has a high Tm and a negative ΔG° (change in Gibbs free energy), indicating the double-strand form was stable[2] and may also be described as thermally stable.

■ Conclusion

Tm values obtained by UV-Vis spectrophotometry were used to calculate changes in the thermodynamic indicators enthalpy, entropy, and Gibbs free energy. These data should provide indicators about the activities of nucleic acids and help improve the effectiveness of drug discoveries and developments.

References
1) Takao Inoue. Development and Market of Biologics 2019, Chapter 4 "Development Trends in Nucleic Acid Drugs", p.33–46 (2019)
2) Chika Akita. Chemical properties of nucleic acid therapeutics: Modifications and functional assessment. Experimental Medicine. Vol.39 No.17, p.74–80 (2021)

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