Experiment 6: Melting Temperature Analysis
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Temperature Effect on Nucleic Acid Stability (Melting Temperature Analysis)

Objective

To evaluate the thermal stability of DNA duplexes and determine their melting temperature (Tm) by monitoring absorbance changes with increasing temperature.

Introduction

Thermal denaturation of DNA occurs when increasing temperature disrupts hydrogen bonding and base stacking interactions, converting double-stranded DNA (dsDNA) into single-stranded DNA (ssDNA). This transition increases absorbance at 260 nm, known as the hyperchromic effect. The melting temperature (Tm)—the point at which half of the duplex becomes denatured—provides valuable information about nucleotide sequence, GC content, length, and ionic strength. Tm analysis is central to PCR optimization, primer design, molecular hybridization, and studies of DNA–ligand interactions.

Principle

As dsDNA is heated, nucleobases become unstacked, increasing absorbance at 260 nm. Monitoring this absorbance across a temperature gradient produces a sigmoidal melting curve. Tm is derived from the curve's midpoint or from the peak of the first derivative (dA/dT). GC-rich sequences typically exhibit higher Tm values due to stronger base stacking and three hydrogen bonds between G and C.

Materials and Equipment

Material / Equipment	**Purpose**
Synthetic complementary oligonucleotides	Preparation of defined duplexes
Tris-HCl buffer	Maintains stable pH
NaCl or $MgCl_2$	Modulates ionic strength and duplex stability
Nuclease-free water	Buffer and dilution preparation
Thermal cycler or heating block	Controlled temperature changes
UV–Vis spectrophotometer with temperature control	Monitoring absorbance vs. temperature
Quartz cuvettes	Heat-resistant optical measurement
Pipettes and filtered tips	Accurate preparation of samples
Microcentrifuge tubes	Duplex annealing and storage
Data acquisition software	Analysis of melting curves

Procedure

1. Prepare equimolar complementary oligonucleotides (1–5 μM) in Tris buffer.
2. Denature at 95°C for 5 minutes, then allow gradual cooling to promote annealing.
3. Transfer the annealed duplex solution into a quartz cuvette.
4. Place the cuvette in the temperature-controlled UV–Vis spectrophotometer.
5. Program a heating ramp (e.g., 10°C → 95°C at 0.5–1°C/min).
6. Record A260 continuously or at defined temperature intervals.
7. Plot absorbance vs. temperature to produce the melting curve.
8. Determine Tm from the midpoint or derivative plot.
9. Compare Tm values under different ionic-strength conditions or sequence designs.

References

1. Bloomfield VA, Crothers DM, Tinoco I. *Nucleic Acids: Structures, Properties, and Functions*. University Science Books.
2. Watson JD et al. *Molecular Biology of the Gene*.
3. Nelson DL, Cox MM. *Lehninger Principles of Biochemistry*.
4. Cantor CR, Schimmel PR. *Biophysical Chemistry*. W.H. Freeman.
5. SantaLucia J. "DNA nearest-neighbor thermodynamics." *PNAS*.

Short Questions (No Answers)

1. How does increasing ionic strength influence DNA melting temperature?
2. Which molecular forces are disrupted during DNA denaturation?
3. Why do GC-rich sequences exhibit higher Tm values?