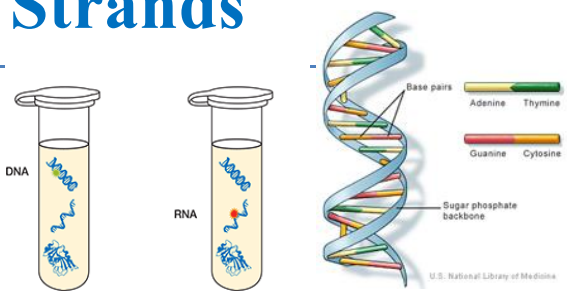
Melting Point (*Tm*) Determination of DNA Double Helix Strands



Objectives (Purposes):

- Are Nucleic Acids dissociated?
- How DNA Strands are melted and Denatured?
- Which Factors effect on denaturation of DNA?
- Why is the DNA double helix so stable (DNA duplex stability)?

- (Purpose) The process of DNA denaturation can be used to analyze some aspects of DNA.
- The compact structure of the double helix (**DNA duplex stability**) is maintained by the **hydrogen bonds** between base pairs and the *van der Waals* interactions between the stacked bases and hydrophobic interactions between the nitrogenous bases and the surrounding sheath of water.
- Various agents (**heat, strong alkalis, urea, and formamide**) weaken such forces and promote the separation of the strands, in a process called **denaturation**. The resulting unwound polynucleotide strands adopt a random arrangement.
- **DNA melting**, also called DNA denaturation, is the process by which double-stranded deoxyribonucleic acid unwinds and separates into single-stranded strands through the breaking of hydrogen bonding between the bases.
- Both terms are used to refer to the process as it occurs when a mixture is heated, although **"denaturation"** can also refer to the separation of DNA strands induced by chemicals like urea.
- **DNA denaturation** can be followed spectrophotometrically by measuring the absorption of UV light at **260 nm**.
- In its native state, DNA absorbs less UV light than the separate polynucleotide chains, a phenomenon that is called **hypochromicity**.
- If a DNA dispersion is slowly heated and its UV light absorption is followed, the temperature/absorbency relationship is an indicator of DNA denaturation (Fig. 1A,B).

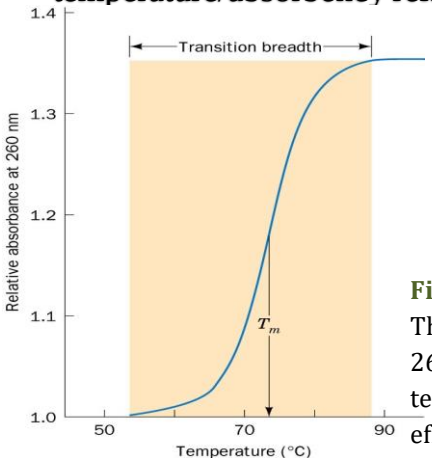


Figure 1A: DNA denaturation. The change in absorbency at 260 nm with increasing temperature (hyperchromic effect) is shown.

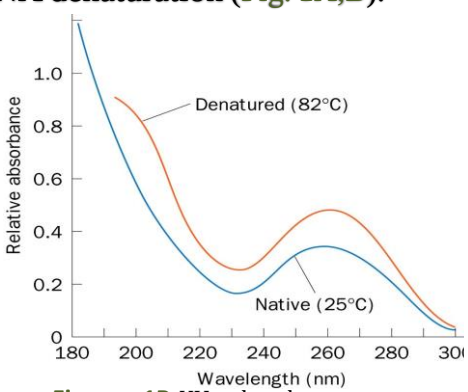
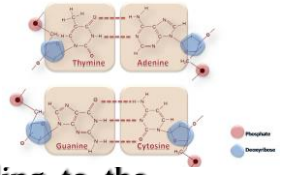


Figure 1B: UV absorbance spectra of native and heat-denatured DNA.

- A sigmoid curve is obtained and as the temperature augments, an increase of absorption (**hyperchromic effect**) occurs. When a given temperature is reached, the optical density does not further increase, which shows that DNA chains are completely separated and the molecules are maximally denatured.
- **Hyperchromicity** is the increase of absorbance (optical density) of a material. The most famous example is the hyperchromicity of DNA that occurs when the DNA duplex is denatured.
- The UV absorption is increased when the two single DNA strands are being separated, either by heat or by addition of denaturant or by increasing the pH level. The opposite, a decrease of absorbance is called **hypochromicity**.
- When DNA denatures, UV abs is due to aromatic bases and increases compared to the double stranded DNA. Results from disruptions of electronic interactions among nearby bases.
- Because **cytosine / guanine base-pairing** is generally stronger than adenosine / thymine base-pairing, the amount of cytosine and guanine in a genome (called the "GC content") can be estimated by measuring the temperature at which the genomic DNA melts. Higher temperatures are associated with high GC content.
- GC-content (or **guanine-cytosine content**) is the percentage of nitrogenous bases in a DNA or RNA molecule that are either guanine (G) or cytosine (C). This measure indicates the proportion of G and C bases out of an implied four total bases, also including adenine and thymine in DNA and adenine and uracil in RNA.

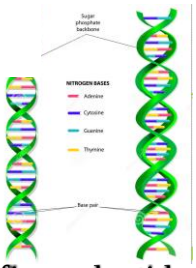


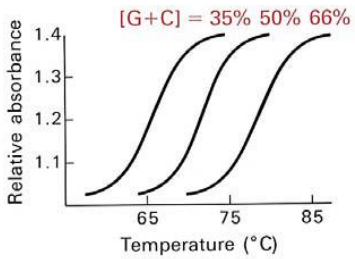
Melting Temperature (Tm):

- The temperature at which half of the DNA is denatured (corresponding to the midpoint or inflection of the curve) is known as the **DNA melting temperature** (Mt).
- The **melting temperature (*Tm*)** is defined as the temperature at which half of the DNA strands are in the double-helical state and half are in the random coil state.
- DNA, when in a state where its two strands are dissociated (***i.e.,*** the dsDNA molecule exists as two independent strands), is referred to as having been denatured by the high temperature.
- The ***Tm*** is characteristic for each DNA under defined conditions of pH and salt concentration, it ranges from 80 to 100°C for DNA isolated from different organisms.
- Melting curves are used to demonstrate the stability of the DNA double helix by estimating the base composition of DNA and determine the melting temperature (***Tm***) which is the midpoint of a melting curve (Figure1).

Tm **is dependent on the followings:**

1. Solvent Solution.
2. Concentrations and types of ions (e.g. Na^+).
3. pH.
4. Mole fraction of GC base pairs.
5. The length of the DNA molecule and its specific nucleotide sequence.





❖ Application of Melting Temperature:

Determining the melting temperature, Tm, is essential for many applications such as PCR, mutagenesis, DNA hybridization, and DNA sequencing.

Denatured DNA can be renatured:

- ✓ The DNA denaturation process is reversible under controlled conditions of pH and ionic strength. If the temperature is slowly decreased in the solution where the DNA had been denatured, the DNA chains will spontaneously reanneal and the original double helix structure is restored and the process called (Renaturation) Figure 2.
- ✓ For example, if a solution of DNA is rapidly cooled below the *Tm*, the resulting DNA is only partially base paired. However, if the temperature is maintained at 25ºC below the Tm, the base paired regions will rearrange until DNA completely renatures.
- ✓ The DNA renaturation resulting from slow cooling (**annealing temperature**) is called annealing condition and it is important for hybridization of complementary strands of DNA or RNA-DNA hybrid double helices.

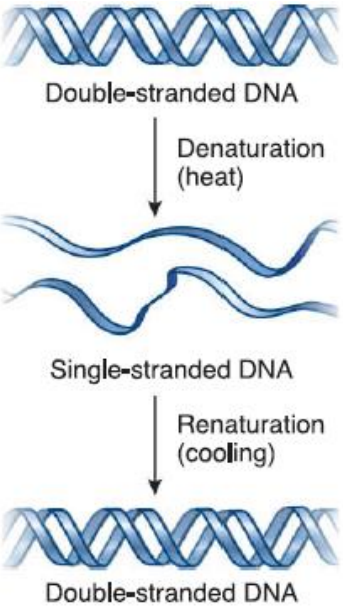


Figure 2: Denaturation and Renaturation of DNA

Procedure:

Materials:

1. **DNA and RNA Solutions.**
2. **Saline-Sodium Citrate (SSC).**
3. **UV spectrophotometer (preferably with temperature control).**

Dissolve your DNA and RNA preparations in SSC to produce a final concentration of approximately 20 µg /ml.

1. Place the dissolved DNA or dissolved RNA in an appropriate quartz cuvette along with a second cuvette containing SSC as a blank.
2. Place both cuvettes into a dual beam temperature regulated UV spectrophotometer and measure the absorbance of the sample at 260 nm at temperatures ranging from 25°C to 80°C. Continue to increase the temperature slowly and continue reading the absorbance until a sharp rise in absorbance is noted.

   Alternatively:

   a) Place the cuvettes into a water bath at 25°C and allow to temperature equilibrate. Remove the blank, wipe the outside dry, and rapidly blank the instrument at 260 nm. Transfer the sample to the spectrophotometer (be sure to dry and work rapidly) and read the absorbance.

   b) Raise the temperature of the bath to 50°C and repeat step (a).

   c) Raise the temperature sequentially to 60°C, 65°C, 70°C, 75°C, and 80°C and repeat the absorbance measurements.

   d) Slowly raise the temperature above 80°C and make absorbance measurements every 2° until the absorbance begins to increase. At that point, increase the temperature, but continue to take readings at 1°C intervals.
3. Correct all of the absorbance readings for solvent expansion relative to 25°C.
4. List the corrected values as At.
5. Plot the value of At /A25 versus temperature and calculate the midpoint of any increased absorbance. This midpoint is the melting point (Tm) for your DNA sample.
6. Calculate the GC content of your sample using the formula: **Percent of G + C = (Tm – 69.3) × 2.44**