RAMA UNIVERSITY

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FACULTY OF ENGINEERING &TECHNOLOGY

DEPARTMENT OF BIOTECHNOLOGY

Absorption of UVlight

- Nucleic acids exhibit characteristic absorption in the ultraviolet region. This absorption is due to the conjugated double bonds and ring system of constituent purine and pyrimidines.
- The more ordered the structure, the less light is absorbed.
- Therefore, free nucleotides absorb more light than a single-stranded polymer of DNA or RNA and these in turn absorb more light than a double-stranded DNA molecule.
- The maximum absorption is at 260 nm (A_{260}) and the minimum absorption is at 230 nm.
- Absorption is proportional to the concentration of the molecule, with a value of 0.02 units per µg DNA per ml.
- For example, three solutions of double-stranded DNA, single-stranded DNA and free bases each at 50 µg/ml have the following A_{260} values:
  - Double-stranded DNA A_{260} = 1.00
  - Single- stranded DNA A_{260} = 1.37
  - Free bases A_{260} = 1.60

  Therefore, double stranded DNA is said to hypochromic and the bases are said to be hyperchromic.

Denaturation of DNAMolecules

- The ordered state of DNA helix, which is, originally present in nature is called the native form.
- The two strands of DNA readily come apart when the hydrogen bonds between its paired bases are disrupted. This can be accomplished by heating a solution of DNA or by adding acid or alkali to ionize its bases. This unwinding of DNA double helix is called melting and a transition from the native to the denatured state is called denaturation.
- Denaturation of DNA molecule can be studied by measuring its absorbance at a wavelength of 260 nm.
- As the DNA is subjected to an increase in temperature, A_{260} starts increasing because of DNA.
- When both the strands are completely separated at a particular higher temperature, there is maximum A_{260} that indicates complete denaturation of the molecule has taken place.
- The temperature at which half of the helical structure of DNA molecule is lost is called its melting temperature (Tm). A convenient parameter to analyze melting transition.
- Molecules rich in GC pairs have a higher Tm than those having abundance of AT base pairs because GC base pairs are more stable and held together by three hydrogen bonds. Such DNA molecules require more energy and hence temperature to denature.

Denaturation involves changes

- Denaturation converts the firm, helical two-stranded native structure of DNA to a flexible, single-stranded denatured state.
- The splitting of DNA molecule into its two strands or chains is obvious because of the fact that the hydrogen bonds are weaker than the bonds holding the bases to the sugar phosphate groups.

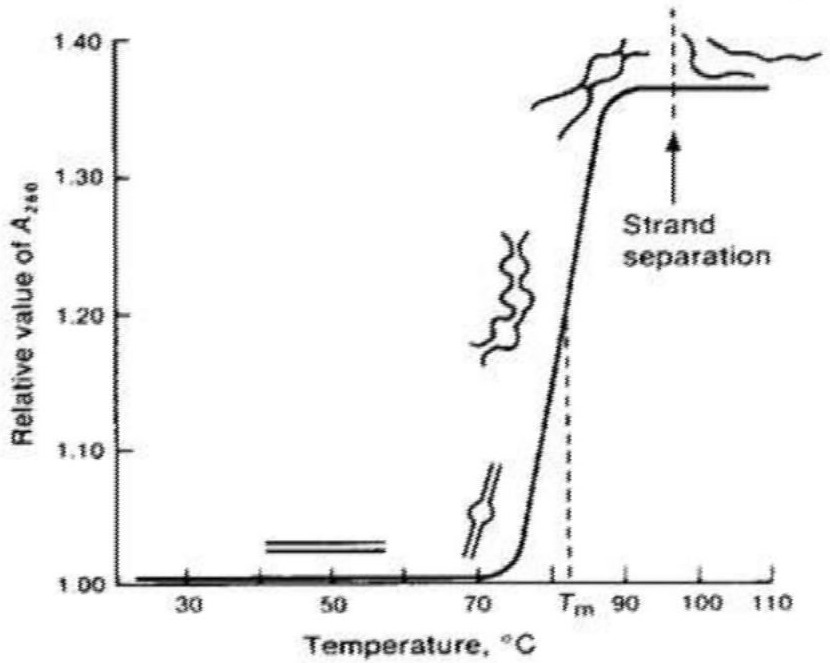
Denaturation involves following changes :

- Increase in absorption of ultraviolet light: Due to resonance, all of the bases in nucleic acids absorb ultraviolet light. And all nucleic acids are characterized by a maximum absorption of UV light at wavelength near 260nm. When the native DNA (which has base pairs similar to a stack of coins) is denatured, there occurs a marked increase in optical absorbance of UV light by pyrimidine and purine bases, an effect called hyperchromicity or hyperchromism which is due to unstacking of the base pairs. This change reflects a decrease in hydrogen bonding.
- Decrease in specific optical rotation: Native DNA exhibits a strong positive rotation which is highly decreased upon denaturation. (same as in proteins)
- Decrease in viscosity: The solutions of native DNA possess a high viscosity because of the relatively rigid double helical structure and long, rodlike character of DNA. Disruption of the hydrogen bonds causes a marked decrease in viscosity

Denaturation and absorbance

- For example (the absorption of ultraviolet light), if a solution of double-stranded DNA has a value of A_{260}=1.00, a solution of single-stranded DNA at the same concentration has a value of A_{260}=1.37.
- This relation is often described by stating that a solution of double-stranded DNA becomes hyperchromic when heated.
- The following features of this curve should be noted:
  - The A_{260} remains constant up to temperatures well above those encountered by most living cells in nature.
  - The rise in A_{260} occurs over a relatively narrow range of 6-8°C
  - The maximum A_{260} is about 37% higher than the starting value

Please note that during melting all covalent bonds, including phosphodiester bonds, remain intact. Only hydrogen bonds and stacking interactions are disrupted.



How can we achieve Denaturation

- Compounds like urea and formamide/formaldehyde are capable of hydrogen bonding with the DNA bases. Hence, they maintain the unpaired state of DNA molecules and result in lowered Tm value, upon melting.
- Formaldehyde reacts with NH_2 groups DNA bases and eliminates their ability to hydrogen bond. Hence addition of formaldehyde causes a slow and irreversible denaturation of DNA.
- There is always a fluctuation in the structure of DNA. The double-stranded regions frequently open to become single-stranded bubbles. This phenomenon is called breathing, which enables specialized proteins to interact with DNA molecule and to read its encoded information.
- Breathing occurs more often in regions rich in AT pairs than in regions rich in GC pairs.
- There are many proteins that can unwind a DNA helix. An example of this type of protein is gene 32 of *E. coli* phage T4, commonly called the 32-protein. This protein binds tightly to the bases of single-stranded DNA. The individual molecules of the 32-protein prefer to line up adjacent to one another along a single strand. Binding of the first molecule is made possible by the breathing of the DNA.
- Denaturation of DNA can also be accomplished by treatment with alkali. Since DNA is quite resistant to alkali hydrolysis, this procedure is the method of choice for denaturing DNA, because heat treatment may often break the phosphodiester bonds and may result in yielding broken fragments of DNA.