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Programme: M.Sc., Biochemistry

Course Title: Chemistry of Biomolecules

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Unit IV

Properties of DNA

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Buoyant density of DNA

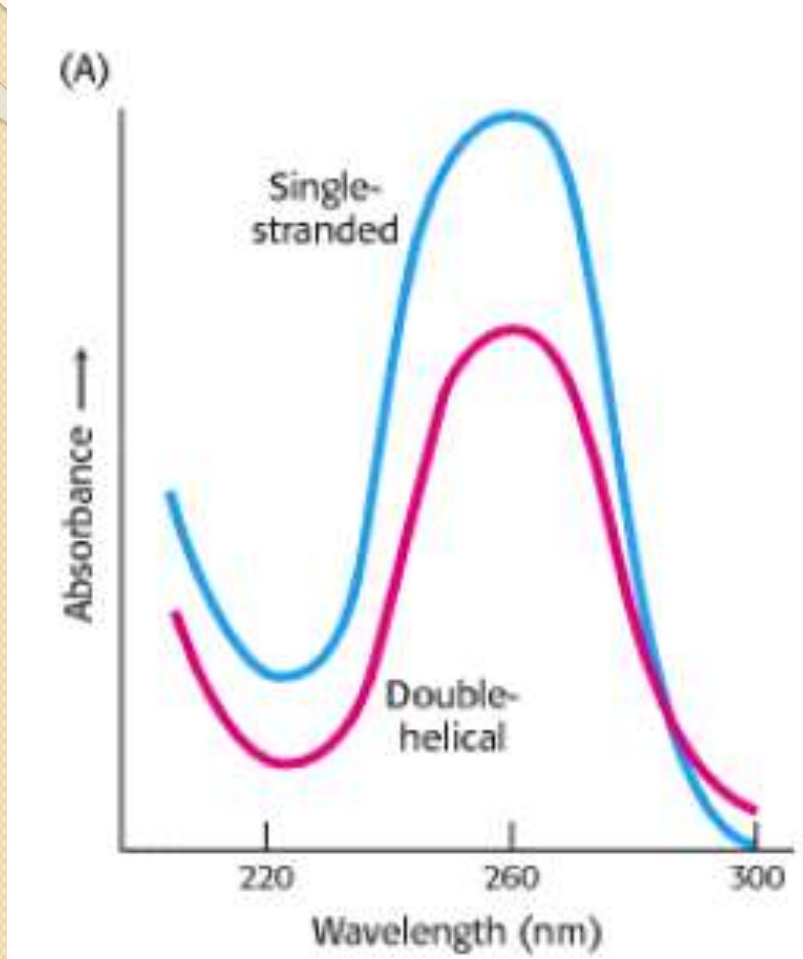
- Density gradient centrifugation is a method used to isolate DNA based on its density. DNA has a density similar to concentrated cesium chloride (CsCl) solutions, (1.6 to 1.8 g/mL, with an average density of around 1.7 g/mL).
- When CsCl solutions are centrifuged at extremely high speeds—where the centrifugal force is 10^5 times stronger than gravity—a density gradient forms within the solution. This gradient results from a balance between the sedimentation of salt ions toward the bottom of the tube and their diffusion upward to regions of lower concentration.
- If DNA is present, it migrates to a position within the gradient that matches its buoyant density, effectively floating at that level. Cesium chloride is chosen for this purpose because its density at 1.6 to 1.8 g/mL is similar to that of DNA.

- The density of DNA is influenced by its G:C content, with G:C-rich DNA having a higher density than A:T-rich DNA.
- Additionally, there is a direct relationship between the buoyant density of DNA from different sources and their G:C content.
- Since DNA and CsCl have similar densities, the DNA settles at a point in the gradient where their densities are equal, forming a sharp, distinct band.

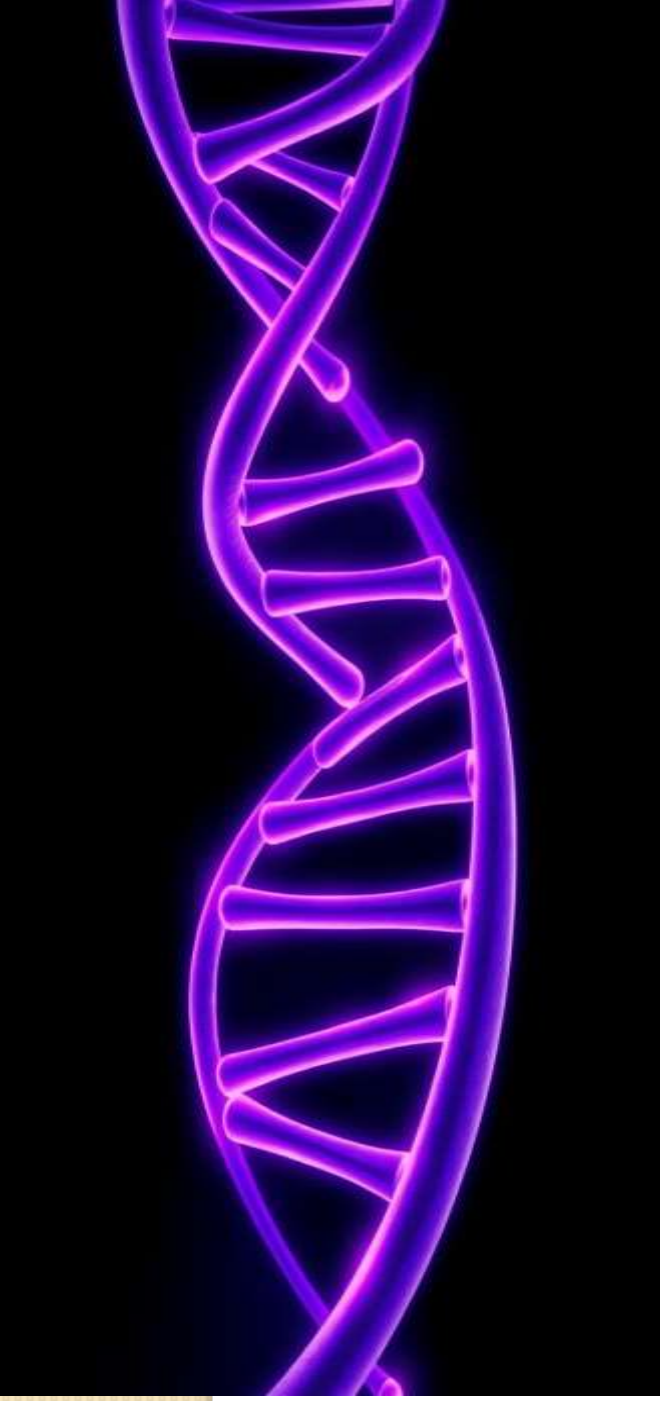
Hypochromism

- The bases of DNA have characteristic absorption maxima and molar absorbancies at 260nm. On the basis of nucleotide composition, absorption of DNA can be predicted assuming that the bases of DNA do not interact.
- However at neutral pH and room temperature, the extinction coefficient per mole of nucleotide in solutions of DNA is only 40% of the value predicted.

- The decrease in the molar extinction coefficient is known as **hypochromism.** This effect is due to the interaction of electrons especially between Pi electrons of the bases at the distance of 3.36A° in the helical stack.
- Stacked bases in nucleic acids absorb less UV light than unstacked bases, an effect called hypochromism.



- Single stranded DNA absorbs light more effectively than double stranded DNA



- Denaturation by Temperature
- Melting Temperature (Tm)
- DNA denaturation occurs when the double helix separates into single strands. This process is triggered by heat, with a characteristic temperature called the melting temperature (Tm) at which 50% of the DNA is denatured.
- G-C Content
- The Tm value is influenced by the DNA's base composition, with higher higher G-C content leading to a higher Tm due to the three hydrogen bonds between guanine and cytosine.

Denaturation - Changes in properties of DNA

- **Hyperchromic** Effect
- Denaturation can be monitored spectro photo metrically by observing the increase in absorbance at 260 nm, known as the hyperchromic effect, due to the unstacking of base pairs.

- **Optical Rotation**
- Double-stranded DNA shows a strong positive rotation which highly decreases with denaturation.

Viscosity

- Viscosity of neutral solutions of DNA is Very high. Denaturation also leads to a decrease in viscosity due to the loss of the rigid double helical structure of DNA.

Denaturation by Chemical Agents

- Urea and Formamide
- Chemical agents like urea and formamide can denature DNA by enhancing the aqueous solubility of purine and pyrimidine bases, disrupting the hydrogen bonds that hold the double helix together.

- Addition of urea lowered T_m value . In 8M urea, T_m is decreased by nearly 20°C. DNA can be completely denatured by 95% formamide at room temperature only.

pH Effects

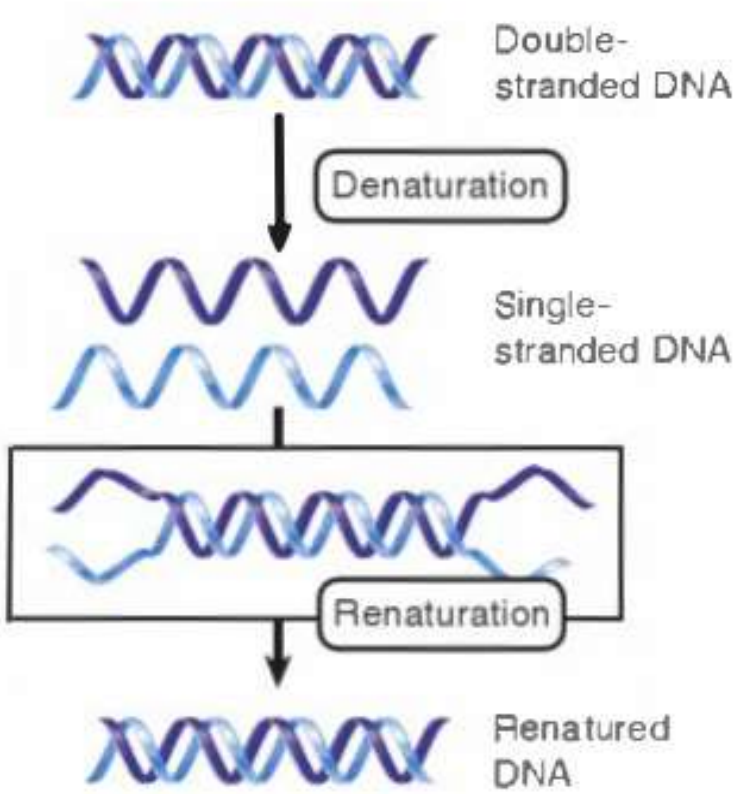
- Extreme pH values can also denature DNA. In acidic solutions,like pH of 2 - 3,protonation of amino groups disrupts the double helix, while in alkaline solutions,pH 12, ionization of enolic hydroxyl groups prevents hydrogen bonding.

Renaturation of DNA

- The denaturation of DNA is reversible under appropriate conditions.
- When denatured DNA is cooled slowly, the separated strands can reassociate, a process called renaturation, It is also called as annealing. The rate of renaturation is influenced by the complexity of the DNA, with repetitive sequences renaturing faster than unique sequences.

- If a heat-denatured DNA solution is cooled slowly (anneling) and hold the solution at about 25°C below T_m and above a concentration of 0.4M Na^+ for several hours, some amount of DNA (50-60%) is renatured. Rapid cooling does not reverse denaturation, but if the cooled solution is again heated and then cooled slowly, renaturation takes place.

Denaturation and Renaturation



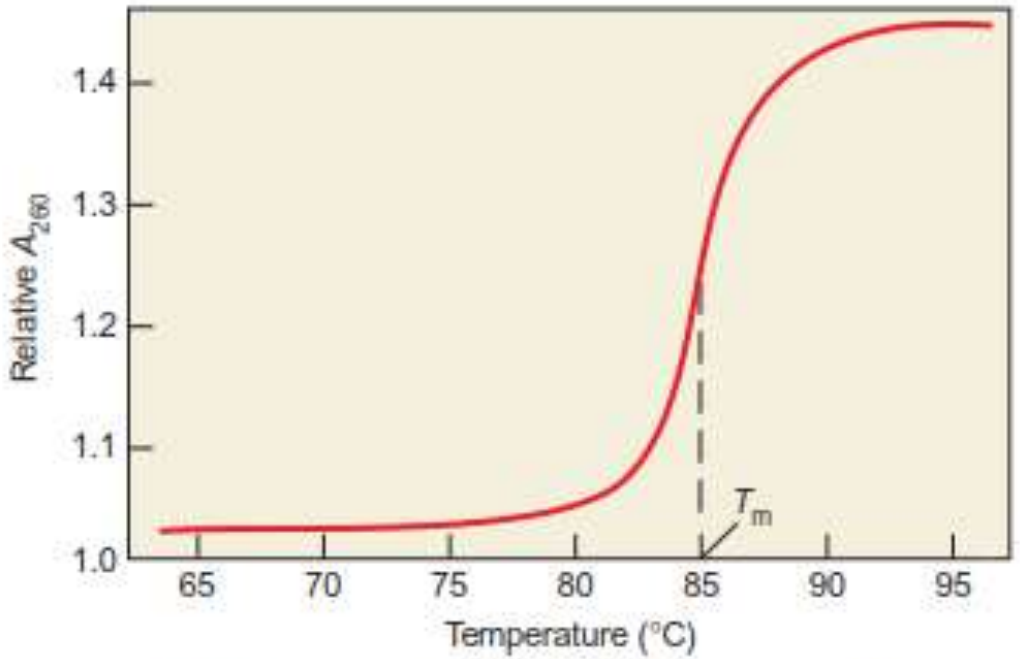
Denatured single strands of DNA can renature to give the duplex form.

Cot curve

- The extent of renaturation over time is influenced by the initial concentration of double-stranded DNA (C_0) before denaturation and the duration of the renaturation process (t), measured in seconds.
- Concentration is typically expressed in nucleotides per unit volume.
- To compare renaturation rates between different DNA samples, the initial concentration (C_0) and the time required to reach half of the maximum renaturation ($t_{1/2}$) are usually measured.
- These values are then multiplied together to calculate a $C_0t_{1/2}$ value. A higher $C_0t_{1/2}$ indicates more complex DNA, which is why λ DNA has a much lower $C_0t_{1/2}$ than human DNA.

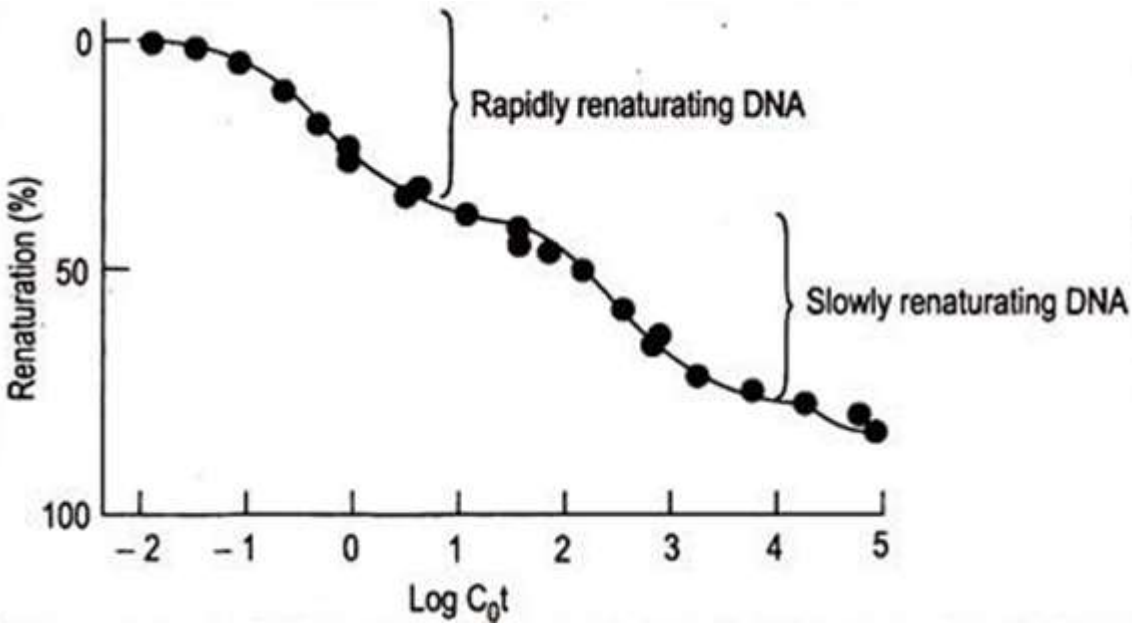
- When the extent of renaturation is plotted against log C_0t (referred to as the Cot curve), it is observed that some DNA sequences renature quickly, while others do so more slowly.
- This suggests that certain sequences are present in higher concentrations than others, implying that parts of the genome consist of repetitive sequences.

DNA melting curve



Melting curve of *Streptococcus pneumoniae* DNA. The DNA was heated, and its melting was measured by the increase in absorbance at 260 nm. The point at which the melting is half complete is the melting temperature, or T_m. The T_m for this DNA under these conditions is about 85°C. (Adapted from P. Doty, *The Harvey Lectures* 55:121, 1961.)

Cot Curve of human DNA



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