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Programme: Bachelor of Science (Third year)

Subject: Botany

Paper Code: BOC 109

Title of the unit: The structures of DNA and RNA/ Genetic material.

Name of the module: Denaturation, Renaturation of DNA, Cot curves and its significance.

Paper Title: Molecular Biology and Genetic Engineering

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Introduction:

DNA has a double stranded helical structure. The stability of this structure is affected by various factors. **Denaturation** is a process in which the **hydrogen bonds** between the **base pairs** of the two strands are broken giving rise to two single strands. However the covalent bonds of DNA remain unaffected. Renaturation is a process which occurs when the denatured DNA strands are cooled under suitable conditions. Renaturation is also known as annealing. DNA denaturation and renaturation processes are used for genetic research and studies.

Denaturation and renaturation kinetics are used to determine the size and complexity of the genome. It is also used to understand the relativity of two genomes and repetitive sequences present in a genome.

Various Methods Causing Denaturation:

1. **Thermal denaturation:** If a DNA solution is heated to approximately 90°C or above there will be enough kinetic energy to denature the DNA completely causing it to separate into single strands. The temperature at which DNA is half denatured is called critical temperature or melting temperature, **Tm**. Tm is dependent on the length and composition of the DNA bases.
2. **Extreme pH:** At high pH (>11.3), hydrogen bonds between base pairs of two strands of DNA dissociate due to presence of **abundant OH^- ion**.
3. **Other Denaturing Agents:** Low salt concentrations destabilise hydrogen bonds. **Formaldehyde and urea** have a tendency to form hydrogen bonds with nitrogen bases. These chemical reagents enhance the aqueous solubility of the purine and pyrimidine groups. **Aldehydes** also prevent hydrogen bonding between base pairs by modifying electronegative centres of nitrogenous bases.
4. **Denaturation by Chemical Agents:**

   Denaturation of DNA double helix can also be brought about by certain chemical agents such as urea and formamide. These chemical reagents enhance the aqueous solubility of the purine and pyrimidine groups. The T_m value is lowered by the addition of urea. DNA can be completely denatured by 95% formamide at room temperature only.

5. Effect of pH on Denaturation:

Denaturation also occurs at acidic and alkaline solutions in which ionic changes of the purine and pyrimidine bases can occur. In acidic solutions at pH values 2-3 the amino groups bind with protons and the DNA double helix is disrupted. Similarly, in alkaline solutions at pH 12, the enolic hydroxyl groups ionize, thus preventing the keto-amino hydrogen bonding.

Effect of Denaturation of DNA:

Increased absorption of UV light at 260nm as the DNA become denatured, a phenomenon known as the **hyperchromatic effect or hyperchromacity or hyperchromism**. This is due to un-stacking of base pairs. The rate of absorption is directly proportional to the rate of denaturation.

Decrease in Specific Optical Rotation:

Double-stranded DNA shows a **strong positive rotation which highly decreases** with denaturation. This change is analogous to the change in rotation observed when the proteins are denatured. **Viscosity decreases**, which reflects the physical change occurred in the DNA structure.

RENATURATION

If melted DNA is cooled it is possible to reassociate the separated strands, a process known as renaturation. However, a stable double-stranded molecule may be formed only if the complementary strands collide in such a way that their bases are paired precisely. But renaturation may not be precise if the DNA is very long and complex. The rate of renaturation can give valuable information about the complexity of the DNA if there are repetitive sequences in the DNA.

it shows less complexity in comparison to its total length, but the complexity is more if all sequences are unique.

Renaturation is also known as annealing. Unwound strands of DNA rewind when the temperature and pH return to optimum biological level, to give back the dsDNA. Renaturation process is fast when DNA is not completely denatured. Renaturation process occurs in a two-step process when DNAs are completely denatured.

a. Complementary strands come together by random collision

b. Rewinding takes place forming a double helix.

Factors Affecting Renaturation:

Renaturation also depends on **temperature, pH, length and constituents of the DNA structure**. The renaturation rate is directly proportional to the number of complementary sequences present.

Effects of Renaturation:

With renaturation absorption of UV (260nm) decreases. Viscosity increases again.

RATE OF RENATURATION

The degree of renaturation is measured by monitoring the decrease in absorbance at 260nm (hypochromic effect) and increase in viscosity. By passing samples at intervals through a column of hydroxylapatite, which retains only double stranded DNAs, how much of the sample is retained is estimated. The rate of renaturation can give valuable information about the complexity of the DNA if there are **repetitive sequences** in the DNA, it shows less complexity in comparison to its total length, but the complexity is more if all sequences are unique.

C_Ot Value

The degree of renaturation after a given time depends on C_0, the concentration of double stranded DNA prior to denaturation, and t, the duration of the renaturation in seconds.

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In order to compare the rates of renaturation of different samples of DNA it is usual to measure C_O and the time taken for renaturation to proceed half way to completion, $t_{1/2}$.Multiply these values together to give a C_O $t_{1/2}$ value.

Define C_Ot value:

C_0t value is thus a product of Co (initial concentration of DNA), t (time in Seconds). and a constant that depends on the concentration of cations in the buffer.The larger the $C_0t1/2$, the greater the complexity of the DNA.

e.g λ DNA has a far lower $C_0t1/2$ than does human DNA.

C_0t curve

The extent of renaturation when plotted against log C_0t, gives a sigmoid curve which is known as C_0t curve. It is a technique to measure the complexity of DNA (size) or genome. This technique was developed by

The technique is based on the principle of DNA renaturation kinetics.

If the extent of renaturation is plotted against log C_0t. It is observed that part of the DNA is renatured quite rapidly while the rest is very slow to renature. This indicates that some sequences have a higher concentration than others i.e., part of the genome consists of repetitive sequences.

These repetitive sequences can be separated from the single-copy unique DNA by passing the renaturating sample through a hydroxylapatite column. At this stage only the rapidly renaturating sequences will be double stranded, and will, therefore, bind to the column. A biochemical technique that measures how much repetitive DNA is in the DNA sample or genome.

Significance of C_0t curve:

It is used to study genome structure and organization. To simplify the genome sequencing that contain large amounts of repetitive sequences.

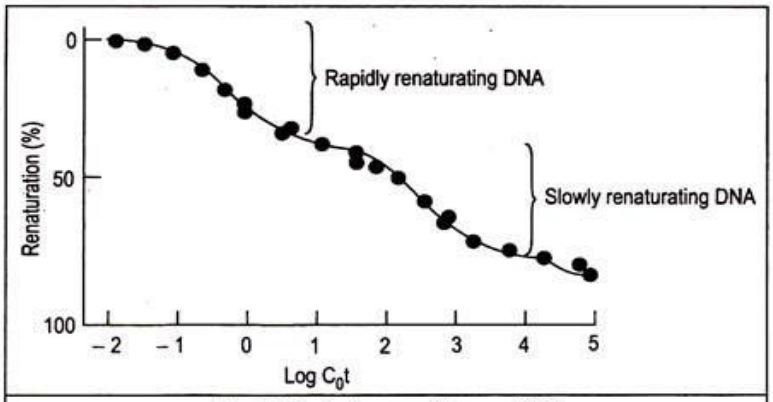

Fig. 3.49 : Cot curve of human DNA