BT 210 Biochemistry Lab

Estimation of Tm of duplex DNA strand using UV-Spectrophotometer

Theory / Principle:

The nucleic acids absorb strongly in the ultra violet region of the spectrum due to the conjugated double bond systems of the constituent purines and pyrimidines. They show characteristic maxima at 260 nm and minima at 230 nm. When duplex DNA molecules are subjected to conditions of pH, temperature or ionic strength that disrupt hydrogen bonds, the strands are no longer held together. The double helix is denatured. If the temperature is the denaturing agent, the double helix is said to melt. As the ordered regions of the stacked base pairs in the DNA duplex are disrupted, the UV absorbance increases. The phenomenon that the relative absorbance of the DNA solution at 260 nm increases as the bases unstack is called hyperchromic shift, which is the result of nearest neighbour base pair interactions. When the DNA is in the duplex state, interactions between the base pairs decrease the UV absorbance relative to single strands. When the DNA is in the single strand state the interactions are much weaker, due to the decreased proximity, and the UV absorbance is higher than the duplex state. If one follows absorbance as a function of temperature, the midpoint temperature of the absorbance curve (also called melting curve) is termed melting temperature Tm **(Fig.1)**.

Fig.1: The effect of temperature on the absorbance of DNA at 260 nm

Methodology:

(a) Materials required:

(i) **Materials:**

- Plasmid DNA
- Milli- Q water
- Eppendorf tubes
- Micropipettes (1000 µl , 200 µl)

(ii)**Equipments:**

- Water bath
- UV Spectrophotometer

Procedure:

1. Take 700 µl of Plasmid DNA in ten different eppendorf tube.
2. Incubate each tube at 10, 20, 30, 40, 50, 60,70 ,80, 90 and 100^{o}C respectively for 10 mins in water bath and immediately chill on ice water.
3. Read absorbance at 260 nm for each sample.
4. Plot a graph of temperature versus absorbance and hence determine the Tm.

(b) Data Analysis:

Melting curves of DNA are commonly described using standard helix-to-coil transition theory. In our case the "helix" is duplex DNA and the "coil" is the disordered single DNA strands. The transition from helix to coil is monitored in our experiment as a function of temperature versus UV absorbance. This can be done because the percentage of hyperchromicity varies linearly with the number of unstacked bases. Thus our melting curve relates the absorbance to the fraction of paired bases (f) as the temperature is increased. The Tm is the temperature where f = 0.5.

References:

1. Puglisi and Tinoco (1989) *Methods in Enzymology* **180**: 304 – 325
2. Breslauer *et al.* (1986) *Proc. Natl. Acad. Sci.* USA **83**: 3746 – 3750
3. Aboul – ela *et al*. (1985) *Nucleic Acids Research* **13(13)**: 4811 – 4824
4. Marky *et al*. (1987) *Biopolymers* **26**: 1601 – 1620
5. David T Plummer (1990) *An Introduction To Practical Biochemistry*, Third Edition, Tata Mc Graw-Hill Edition