Determination of G+C % rate & melting temperature

Although the ratio of G to C and A to T in an DNA organism is fixed , the GC content (percentage of G+C) can vary considerably from one DNA to another . This character is very important in determination the genetic similarity among living organism and widely used in molecular biology techniques and genetic analysis to estimate the genetic distance between two species ; e.g : PCR , DNA/DNA and DNA/RNA hybridization .

As molecules , DNA is considered a thermo-stable molecule up to 70 C° due to many structural factors although when a DNA solution is heated enough , the non-covalent forces (hydrogen bonds) that hold the two strands together weaken and finally break .

When this happen , the two strands cone apart in a process known as DNA denaturation or DNA melting . The temperature at which the DNA strand are half denatured is called the melting temperature (Tm)

The melting temperature (Tm): is defined as the temperature at which 50% of the DNA exists as a double and 50% is single strands .

The melting temperature (Tm) of DNA sample depends on many factors , including :

1-The hydrogen bond within the structure

2-Proportion of GC pairs , because the three hydrogen bonds between GC pairs are more stable than the two between AT pairs .

The melting point of DNA sample hence give information about its base composition (G/C content).

In double helix of DNA , the nucleotide are complementary in another words , G=C , A=T.

Measurement of DNA G-C % ratio by melting curve analysis :

The melting temperature (Tm-value) of DNA can be easily detected by measuring the absorption at 260 nm . The dissociation of the base pairs brings about an increase in absorption . This is called the hyper chromatic effect . Alteration of secondary DNA structure upon rising of the temperature results in change of light a absorption . For example if a solution of a double –stranded DNA has a value of A260=1.00 , a solution of single –stranded DNA at the same concentration has a value of A260=1.37. This relation is often described by stating that a solution of double –stranded DNA becomes hyper chromatic when heated .The differences related to the facts that the DNA helix is stacked .When the DNA Sample is gradually cooled the separated strands are renaturated so the absorbance of this sample will reduce due to hypo chromatic shifts .

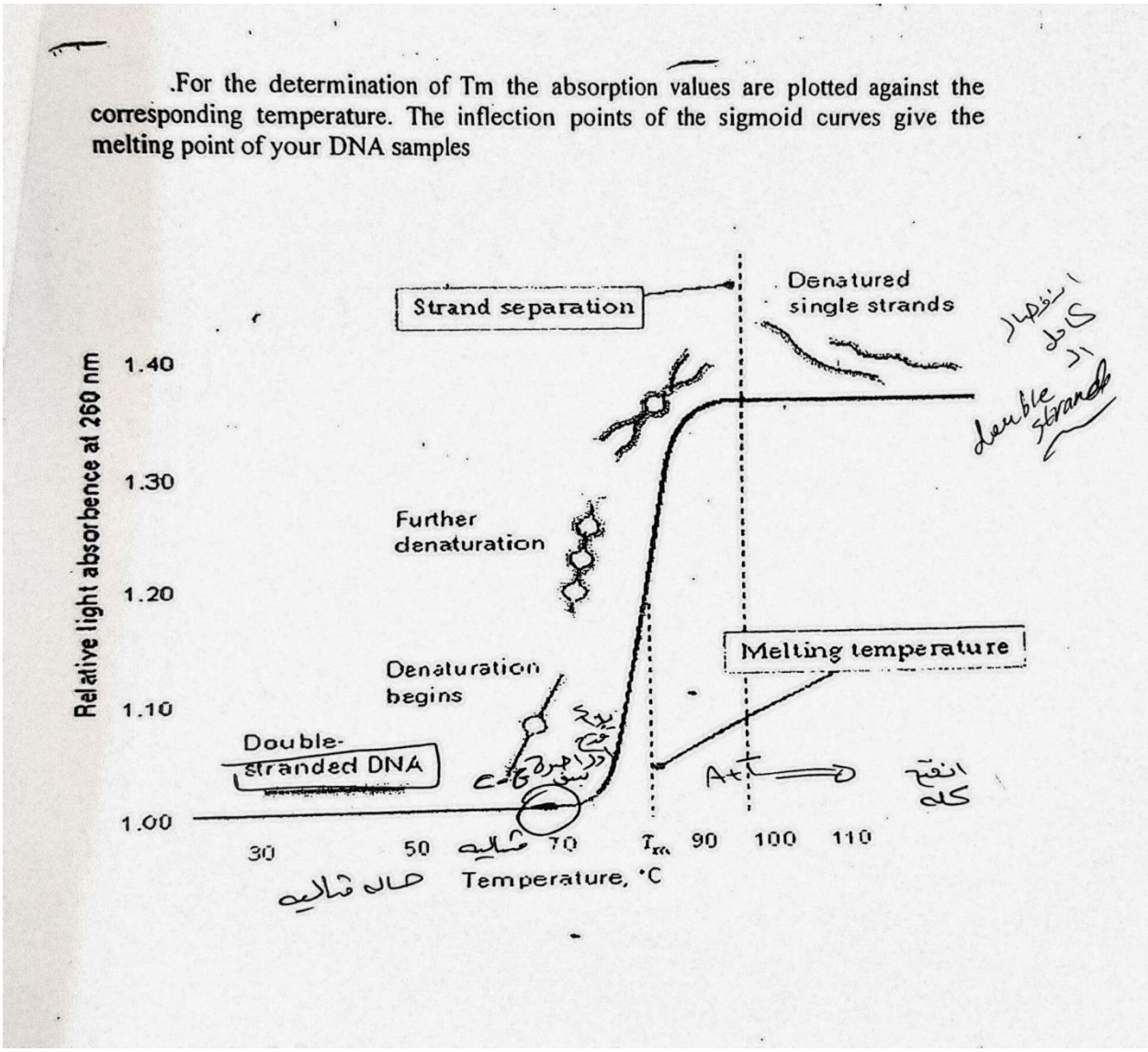
Methodology

1-suspend the DNA sample in TE buffer to obtain 20 µg/ml working concentration

2-Reset (zero) the spectrophotometer device on 260 nm by TE buffer as blank .

3-Gradully heat the DNA sample in water bath and measure the absorbance of sample at 50,60,70,80,90,100 C°

4-Draw the DNA melting curve by plotting the absorption values against the corresponding temperature .The inflection point of the sigmoid curves give the melting point of your DNA samples



Calculate the mol G+c % by using the following formula :

*Tm=70+ (0.47 X G+C %)

*G+C % of a DNA sample =(TM-70)x 2.5

*calculate the temp. of renaturation (TOR) by using the following formula

TOR = 52 +(0.47 X G+C %)

*Tm =TOR +18

Example : calculate the Tm and TOR of a DNA sample contain 10% A (adenine)

Determination of DNA ,RNA purity :

After a DNA purification procedure we often want to determine the quantity (concentration) of the DNA solution and to evaluate the purity .

Both quantity and quality can be determined using spectrophotometer . DNA absorbs light in the UV range (260 nm) , Therefore when light of this wavelength shines through a sample of DNA , the amount of light absorbed is proportion amount of DNA is the solute .

The absorbance of light is called optical Density (O.D) .The concentration of DNA can be calculate using the following formula

1 O.D 260 unit =50 µg/ml double stranded DNA

1 O.D 260 unit of RNA =40 µg/ml RNA

We are usually more interested in the amount of protein that might have been inadvertently purified with the DNA. Protein absorbs light at a different wavelength (280 nm) . A convenient measure of DNA purity is determined by measuring the O.D 260/O.D 280 ratio .

Pure DNA (free of protein contamination) will have an A 260 /A280 . ratio close to 1.8 . If the phenol or protein contamination is present in the DNA preparation , the A260/A280 ratio will be less than 1.8 . If RNA is present in the DNA prep , the A260/A280 ratio may be greater than 1.8

*Pure RNA preparation will have an A260/A280 ratio close to 2.0

*concentration =(O.D 260 X 50 µg/ml x dilution factors)

*Purity of DNA sample =O.D 260/O.D 280

We can determined the amount of contaminated protein :

Contaminated protein (mg/ml)=(1.55*O.D . 280)-(0.76 O.D. 260)

There are several factors that may affect A260/A280 ratios .

*Different instrument

*Concentration of nucleic acid sample

*The types of protein present in a mixture of DNA and protein

Example :

If you measure the absorbance of DNA sample at 260 and 280 and obtain the following readings :

O.D 260 =0.550

O.D 280=0.324

Calculate the purified DNA sample and the concentration of contaminated proteins

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