application note

Thermal Analysis of DNA by UV/Visible Spectrometry

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Introduction

Deoxyribonucleic acids (DNA) are chain-like macromolecules that have an important function in the storage and transfer of genetic information, and are involved in protein synthesis in living cells.

DNA consists of two polynucleotide chains arranged in a helical structure. Each nucleotide is composed of three constituent parts: a phosphate group, a five carbon sugar (deoxyribose), and an organic nitrogen-containing base. Four different kinds of nucleotides occur in DNA, differing in the nitrogenous bases. They comprise the purines, adenine and guanine, and the pyrimidines, cytosine and thymine. The nucleotides within DNA are bonded together such that the sugar of one nucleotide is always attached to the phosphate group of the next nucleotide. The nitrogenous bases are orientated as side groups off the chains. The two chains of DNA are loosely bonded together by hydrogen bonds between adenine and thymine and between guanine and cytosine from opposite chains. These hydrogen bonds provide the thermodynamic stability of double-stranded DNA in solution.

When solutions of DNA are exposed to extremes of pH or heat or to solutes such as urea or amides, the double helical structure of DNA undergoes a transition into a randomly single-stranded form known as denatured DNA. During denaturation the interactions between successive base pairs are interrupted. When DNA denatures, significant changes occur in a number of its physical properties, such as an increase in buoyant density, decrease in viscosity and an increase in the UV absorption at 260 nm. This last effect is known as the hyperchromic effect and provides a convenient method for monitoring the denaturation of DNA.

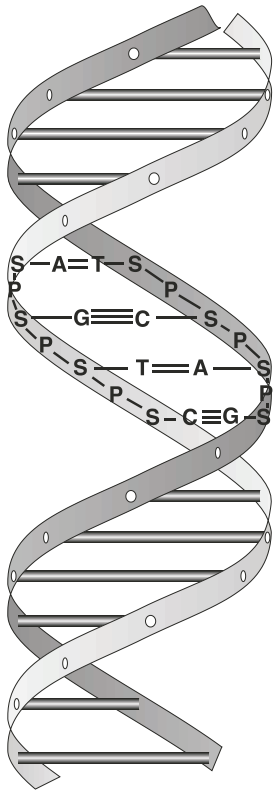

Fig. 1. . Schematic of the double helix of a DNA molecule showing A-T and G-C bonding pairs. P indicates a phosphate ester, S means deoxyribose sugar, A = T is the adenine-thymine pairing, and G ≡ C is the guanine-cytosine pairing. After Conn and Stumpf.'

The most common method of studying denaturation of DNA involves increasing the temperature of a DNA solution so that the DNA gradually denatures by strand separation. A UV/Visible spectrophotometer with a temperature control accessory is ideal for such studies as the temperature can be readily controlled and monitored and the denaturation can be monitored by observing absorption changes at 260 nm.

In this study a specific software application program for GBC double beam UV-Visible instruments was used to investigate thermal denaturation for three types of DNA. For one of the DNA samples, *Escherichia coli,* renaturation behaviour was also studied.

Experimental

Reagents/Materials

DNA from *Escherichia coli* Strain B *(E. coli), Clostridium perfringens* and calf thymus were obtained from Sigma Chemical Company. DNA solutions (typically 20 µg/mL) were prepared by dissolving the DNA samples in standard saline citrate buffer (SSC) or a one in ten dilution of standard saline citrate buffer (0.1 SSC) with continuous stirring for 24 hours. SSC is a buffer consisting of 0.15 M sodium chloride and 0.015 M sodium citrate, at pH 7.0. The diluted buffer is used to ensure completion of the denaturation without boiling the solution.

Instrument

A GBC 918 double beam UV/Visible spectrometer running "DNA MELT" application software was used in this study. The instrument was fitted with a Peltier-effect thermostatted cell holder accessory complete with sample immersion probes. The sample immersion probes allow accurate measurement of the actual solution temperature instead of the surrounding block temperature. The "DNA MELT" software allows full computer control of the Peltier device. The thermal denaturation temperature, Tm, may be automatically calculated at the completion of a run using either a mid-point graphical method or first derivative method. The guanine-cytosine content (GC%) is calculated from the T_m using the standard Marmur equations[2] or a user-defined equation which relates Tm to GC%.

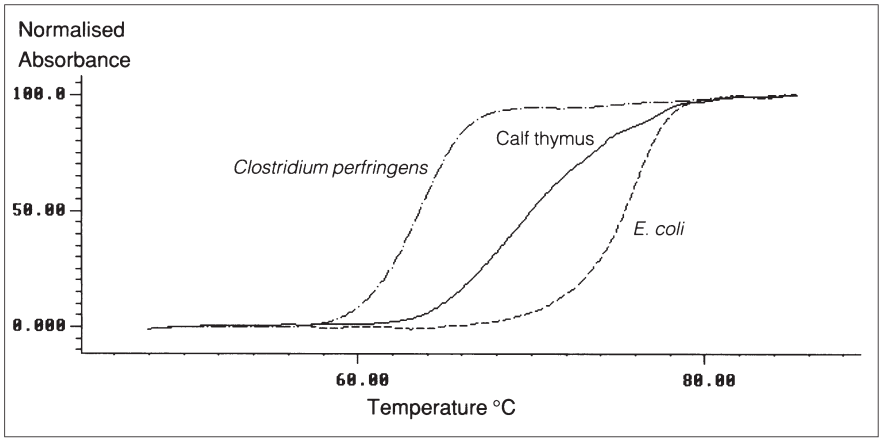

Fig. 2. Thermal melt curves for three DNA types in 0.1 SSC

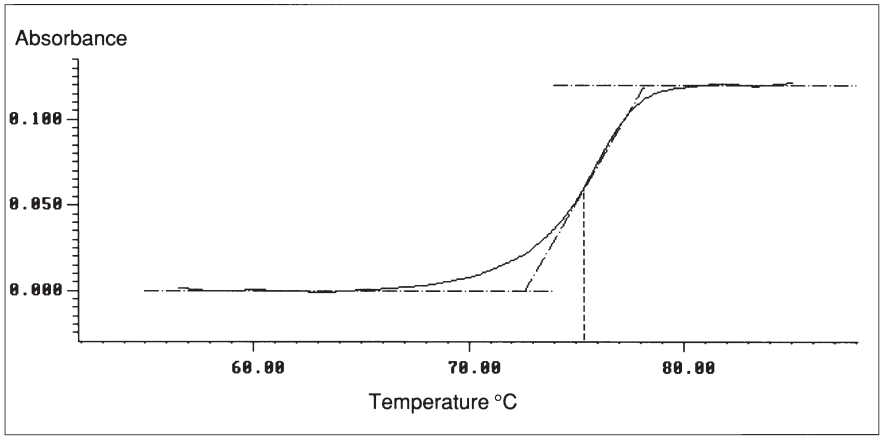

Fig. 3. Mid-point determination of T_m for E. coli.

The instrument parameters used in this study are given in Table 1.

Table 1. Instrument operating Parameters

Measurement wavelength: 260 nm			
Measurement time 5 s			
Time between measurements: 12 s			
Temperature program:			
Step	Temperature °C	Ramp rate (°C/min)	Hold time (min)
1	50	10	2
2	90	0.5	0

Results

Figure 2 shows the thermal denaturation curve (absorbance versus temperature) for the three DNA types in 0.1 SSC obtained at a temperature ramp rate of 0.5°C/min.

Characterisation of thermal denaturations is based on the determination of T_m (the thermal denaturation temperature). The T_m is defined as the mid-point of the thermal transition curve. The mid-point can be determined graphically as shown in Figure 3, or by locating the maximum in the first derivative of the thermal transition curve, Figure 4.

Table 2. Thermal denaturation data (°C) for three DNA types.

DNA type	Observed T_m in 0.1 SSC	Corrected T_m for SSC	Literature value
E.Coli Strain B	76.0	91.4	90.5
Calf thymus	70.4	85.8	87.0
Clostridium perfringens	63.7	79.1	80.5

The observed T_m values for the three DNA types in 0.1 SSC using the derivative method are given in Table 2. These T_m values can be corrected to correspond to T_m values in SSC via the relationship described by Mandel and Marmur[3]:

$$T_m\,(SSC) = T_m\,(0.\ 1\ SSC) + 15.4°C \ldots . (1)$$

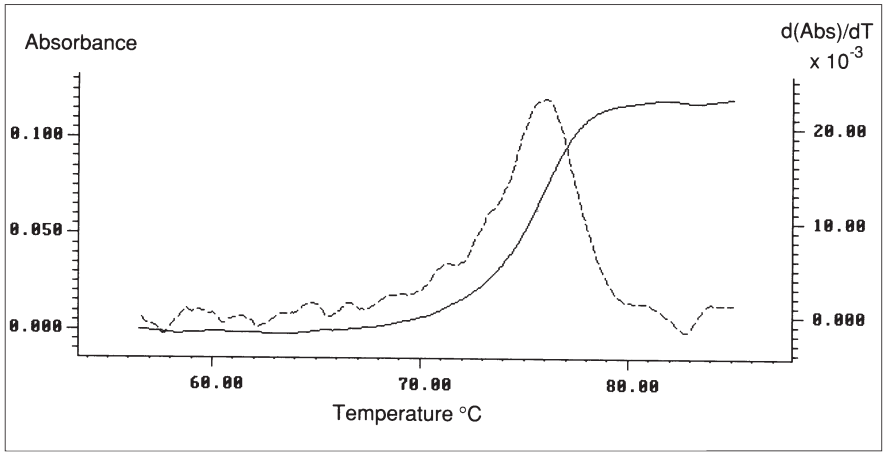

Fig. 4. Determination of T_m using the first derivative curve (broken line) for E. coli.

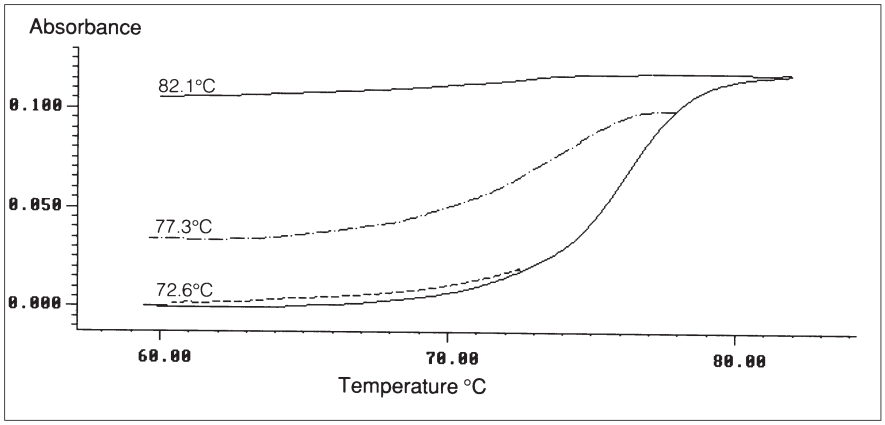

Fig. 5. Thermal renaturation curves for E. coli. following denaturation at three temperatures.

The corrected T_m values and the reported literature values are also given in Table 2. Good agreement is obtained between the literature values and the corrected T_m results.

The T_m value of a DNA species is dependent upon the proportion of G-C base pairs, which have three hydrogen bonds. The higher the content of G-C pairs, the more stable the structure and the higher the T_m value. Marmur[2] has determined an empirical relationship between G-C content in DNA and the observed T_m for DNA in SSC:

$$GC\% = 2.44 (T_m - 69.3) \quad \ldots\ldots\ldots \quad (2)$$

From equations (1) and (2) the relationship between GC% and the T_m for DNA in 0.1 SSC can be obtained:

$$GC\% = 2.44 (T_m - 53.9) \quad \ldots\ldots\ldots \quad (3)$$

The GC% values for DNA from *E. coli* Strain B, calf thymus and *Clostridium perfringens* were 54%, 40% and 24%, respectively. These values are in good agreement with published values.'

The denaturation of DNA is a gradual process. Initially the two strands partially separate but remain united by a short segment of double helical structure. Strand separation continues until the two strands are completely separated. Denaturation of DNA is reversible if the two strands have not been completely separated, but the renatured strands will probably not have the original base pairings.

Thermal renaturation involves monitoring the absorbance of denatured DNA in a controlled cooling experiment. In this study *E. coli* Strain B (in 0.1SSC) was denatured at 3 temperatures, 72.6°C, 77.3°C and 82.1°C, and then the absorbance at 260 nm monitored whilst cooling the DNA solution. ne resulting thermal renaturation curves are shown in Figure 5.

When the *E. coli* Strain B DNA was denatured at 82.1°C only a small decrease in absorbance was observed during cooling, indicating that the DNA strands had almost completely unwound. When denaturated at 77.3°C the absorbance during cooling decreased significantly but still remained higher than the original, indicating significant unwinding, but probably not complete separation, of the DNA strands. Finally, when denatured at 72.6°C, the DNA sample had undergone only partial strand separation, as indicated by the absorbance during renaturation returning to a value only slightly higher than the original.

Conclusion

The GBC range of double beam UV-Visible spectrometers provides a simple and versatile means of studying the denaturation and renaturation of DNA solutions. The DNA MELT software gives complete flexibility in data handling and storage. Determination of T_m can be performed either by a mid-point graphical method or from the maxima of the first derivative, whilst the GC% can be determined from standard or user-defined equations. All raw data and calculated results are automatically saved to disk and may be recalled for further post-run manipulation or recalculation. The Peltier-effect thermocell is completely computer controlled and the software allows up to 10 steps, each with user-defined ramp rate and hold time, in the temperature program. The user can be assured of accurate transition temperatures by the use of the sample immersion probes that allow measurement of the actual sample solution temperature. Finally, the use of a multi-sample temperature accessory allows the simultaneous determination of up to 12 samples for increased sample throughput.

References

1. Conn, E.E. and Stumpf, P.K.; Outlines of Biochemistry, 4th Edition, John Wiley & Sons, Inc., NY, (1976).

2. Marmur,J. and Doty, P.;J. Mol.Biol. Vol5, 109, (1962).

3. Mandel, M. and Marmur, J.; *Methods Enzymol.* Vol 12 Part B, 195 (1968).