SHIMADZU
Excellence in Science

Application News

No. UV-013

Spectrophotometric Analysis

Thermal Analysis of DNA using the Shimadzu TMSPC-8 Temperature Controlled Accessory

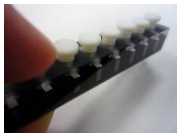
Obafemi Ajayi, Mark Talbott, John Kinyanjui, Jeff Head

■ Introduction

The study of DNA is central to the understanding of many biological processes. DNA is a polymer made up of nucleotides containing four major bases: adenine, thymine, guanine, and cytosine. These nucleotides are held together by 3', 5'-phosphodiester bonds to form single-stranded DNA (ssDNA). The important double stranded DNA (dsDNA) is the combination of two ssDNA strands, which are held together by hydrogen bonds where adenine (A) is always bound to thymine (T) and guanine (G) is always bound to cytosine (C). The AT bond is comprised of two hydrogen bonds where the GC pair is bound by three hydrogen bonds and is, therefore, a stronger bond.

DNA exhibits absorption properties in the UV range at 260 nm because of the presence of these aromatic bases. This feature provides useful information into the base-pair composition of DNA because structural changes such as helix unwinding affect the extent of absorption[1].

If dsDNA samples are treated with denaturing agents such as heat, alkali, or organic solvents, the H-bonds can be broken and the absorbance values at 260 nm will change. For example, Figure 1 shows a typical thermal denaturation curve for DNA, which shows changes in absorbance as the temperature of the sample is increased. The total increase in absorption for this type of analysis is usually on the order of 40% and occurs over a small temperature range. The temperature corresponding to the midpoint of the absorption increase is defined as T_m, the transition, or melting temperature. The T_m is the temperature where 50% of the base-pair H-bonds in the dsDNA no longer hold the two strands together.



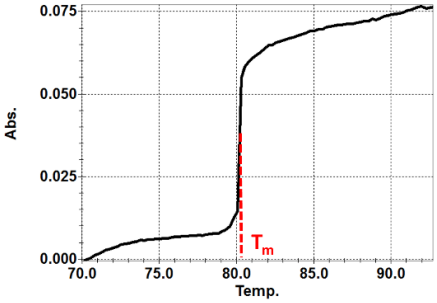


Figure 1: Typical thermal denaturation curve for DNA.

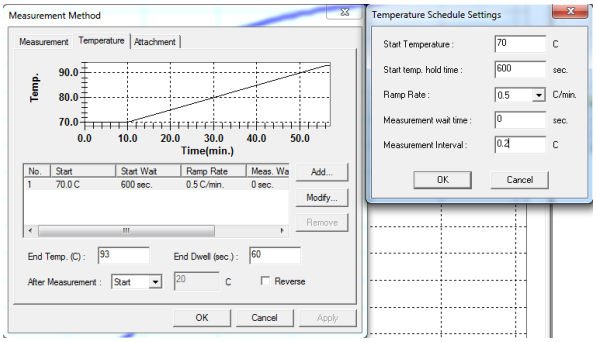
T_m is an important physical constant that can be used in the identification and characterization of DNA. The higher the T_m value, the greater the guanine plus cytosine content (%GC pairs) and the more stable is the double helix[2].

The relationship between the T_m value and the %GC is given by the following equation,
%GC = 2.44 (T_m-69.3)

The Thermal Melt Analysis System for Nucleic Acids (TMSPC-8), along with the T_m Analysis software offered by Shimadzu, presents the ideal solution for determining the T_m value of a DNA solution. The versatility of this system allows for setting multiple ramp rates in a single acquisition, storing data files and routine methods for recall in future analysis, as well as providing the user with the ability to select from various algorithms for calculating the T_m values for previously stored runs. The purpose of this application news is to demonstrate the measuring capabilities of the Shimadzu TMSPC-8 temperature controlled micro cell holder for determining the T_m values of various DNA samples.

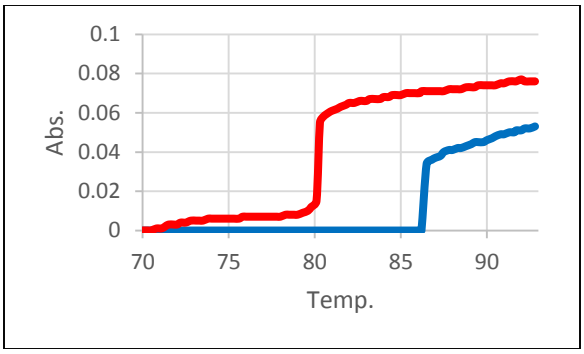
■ Experimental

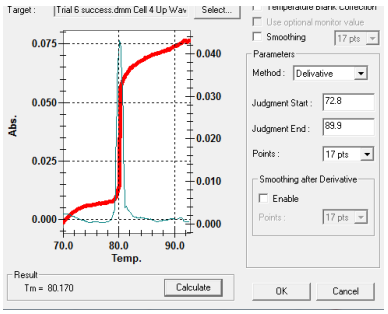
Two separate solutions were prepared by dissolving the calf thymus DNA in Dulbecco's Phosphate Buffered Saline (DPBS) and Tris-EDTA buffer solution, resulting in a concentration of 2 mg/mL. For analysis, 100µL of sample were pipetted into an 8-series micro cell which fits inside of the TMSPC-8 temperature controlled accessory. A Shimadzu UV-1800 spectrophotometer equipped with the TMSPC-8 was used for the thermal melt experiment. Using a ramp rate of 0.5°C/min, the absorbance of the sample was recorded at 260 nm between 70 and 93°C. The cuvette and peltier block were allowed to equilibrate at 70°C for 10 minutes prior to data acquisition. The absorbance of the DNA was recorded at temperature intervals of 0.2°C with a slit width of 1 nm. The T_m was calculated using the preprogrammed fitting methods offered in the T_m Analysis software.



■ Results and Discussion

Figure 2 shows the thermal denaturation curves for the calf thymus DNA suspended in DPBS and Tris buffers. The choice of buffer used for thermal analysis studies can impact the determined T_m value, as demonstrated in this application news.





DNA/Buffer Combination	Tm Value	%GC
Tris Buffer (Blue)	86.4	41.7
DPBS Buffer (Red)	80.2	26.6

Figure 2: Thermal denaturation curves of calf thymus DNA suspended in DPBS buffer (Red) and Tris buffer (blue), along with determined T_m values using the derivative method.

■ Conclusion

In conclusion, the purpose of this application news was to demonstrate the capabilities of the Shimadzu Thermal Melt Analysis software for determining the T_m values of various DNA solutions. The UV-1800 when combined with the TMSPC-8 Thermal Melt Analysis System is capable of monitoring the change in absorbance of dsDNA melting as a function of temperature and determining T_m values for the dsDNA/buffer combination used. With the use of an 8-channel micro cell, multiple thermal denaturation curves can be collected simultaneously for easy comparison and routine analysis.

■ References

1. Boyer, Rodney F. "Modern Experimental Biochemistry". Second Edition. The Benjamin/Cummings Publishing Company. 1993.
2. UV Talk Letter. Volume 7. SHIMADZU. C101-E120.

First Edition: October 2014

SHIMADZU Corporation
www.shimadzu.com/an/

SHIMADZU SCIENTIFIC INSTRUMENTS
7102 Riverwood Drive, Columbia, MD 21046, USA
Phone: 800-477-1227/410-381-1227, Fax: 410-381-1222
URL: www.ssi.shimadzu.com

For Research Use Only. Not for use in diagnostic procedures.
The contents of this publication are provided to you "as is" without warranty of any kind, and are subject to change without notice. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication.

© Shimadzu Corporation, 2014

Related Solutions

- Price Inquiry
- Product Inquiry
- Technical Service / Support Inquiry
- Other Inquiry