

Determination of DNA and RNA Melting Point on UV-VIS Photometer SPECORD® PLUS

Alexandra Kästner, Molecular Spectroscopy, Analytik Jena AG, 07745 Jena, Konrad-Zuse-Str. 1

Nucleic acids are biological molecules essential for life. Together with proteins nucleic acids make up the most important macro molecules where each is found in abundance in all living things. They allow organisms to transfer genetic information from one generation to the next. There are two types of nucleic acids: deoxyribonucleic acid, better known as DNA and ribonucleic acid, better known as RNA. Their names are derived from type of sugar, ribose, contained within the molecules. In living organisms, DNA does exist as a double helix structure. The stability is achieved by stacking and the hydrogen bonds between the bases attached to the two strands. The four bases found in DNA are adenine (abbreviated A), cytosine (C), guanine (G) and thymine (T). They form complementary pairs: The nucleotides hydrogen bond to another nucleotide base in a strand of DNA opposite to the original. This bonding is specific, and adenine always bonds to thymine (and vice versa) and guanine always bonds to cytosine (and vice versa).

Variations in pH or heating can affect to a structure modification of DNA, where the double-stranded deoxyribonucleic acid unwinds and separates into single-stranded strands through the breaking of hydrogen bonding between the bases. This denaturation process is called as DNA melting and leads to increasing of absorbance (hyperchromic effect).

From there one of the most commonly used and simplest techniques for the DNA melting point determination is spectroscopic determination by UV absorption. The absorption spectrum is recorded against the dependence of the temperature where the turning point of the graph describes the exact melting point. The temperature where at the half of the DNA exists as single strands, is called melting point (T_m).

Theory

All nucleic acids absorb strongly in the UV region due to the heterocyclic ring structure associated with each of the four bases. Typically absorption maximum is observed at a wavelength of around 260nm, although this is pH dependent. The versatility of DNA comes from this fact that the molecule is actually double-stranded. The bonding between cytosine with guanine is generally stronger than adenosine/thymine base-pairing. [1]

The amount of cytosine and guanine (called the 'GC content') can be estimated by measuring the temperature at which the DNA melts. Higher temperatures are associated with a high GC content. So by melting point determination it is possible to classify bacteria because the GC content in the DNA is an important fact of any organism. [2]

DNA denaturation can also be used to detect sequence differences between two nucleic acids of different origin. DNA is heated and denatured into single-stranded state, and the mixture is cooled to allow strands to rehybridise. Hybrid molecules are formed between similar sequences and any differences between those sequences will result in a disruption of the base-pairing [3].

In field of disease research rare gene mutations can be detected because mutated DNA sequences melt at lower temperatures than 'normal' ranges. Furthermore the process of DNA melting plays an important role in molecular biology techniques, notably in the polymerase chain reaction (PCR).

Experimental

DNA melting point determination is performed on SPECORD® 200 PLUS by means of the peltier temperature cell holder. SPECORD® 200 PLUS is a real double beam photometer for the wavelength range of 190-1100nm. It has a fixed spectral bandwidth of 1.4nm and two photodiodes. [4]

The peltier temperature accessory enables applications that demand high temperature accuracies like accurate protein analyses and examination of photochemical reactions or like this application the determination of DNA melting point. Temperature control of the cell holder is performed via a separate control unit and can be regulated in a temperature range of -5 to 105°C and with an accuracy of $\pm 0.1^\circ\text{C}$ (Figure 1). The controlling sensor used is a measuring sensor located at the outer bottom corner of the cell block. In addition to the controlling sensor, the cell holder contains two further sensors for optional monitoring of either the holder or the cell temperature. The cell sensor is specially designed for ultra-micro cells with round PTFE stopper. It may remain in the cell during the analytical measurement (Figure 2). [5]

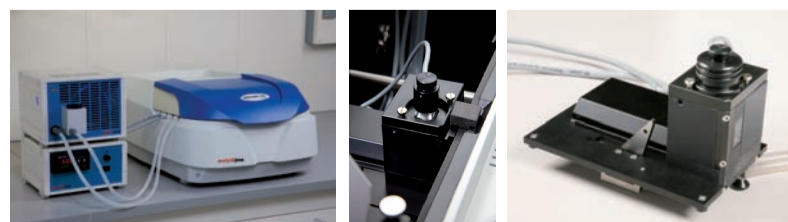


Figure 1. (a,b,c) SPECORD® PLUS with peltier temperature cell holder

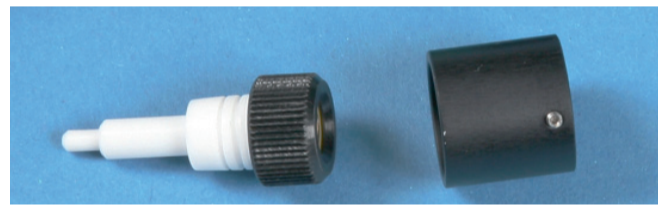


Figure 2. Cell sensor for ultra- micro cells

Sample material

Two different DNA samples were analysed for DNA melting point determination. The first sample was Plasmid DNA from bacteriophage Lambda. Plasmid is a circular double strand DNA molecule that is not integrated into bacterial chromosome.

The second sample was an animal DNA that is from a thymus of a calf.

Lambda DNA stock solution was diluted with water for the determination of plasmid DNA. The optimum dilution is achieved when the absorption maximum lies between 0.1 and 1 Abs. The spectrums of the samples are taken for this purpose between 220nm and 300nm and the peak maximum has been found (Figure 3). The analysis of a 100µL DNA solution was performed on an ultra-micro cell against molecular water used as a blank solution. The final melting point measurement was carried out in 'simultaneous' mode between the temperature range of 25°C to 70°C and at 260nm, where the spectrum of DNA has a maximum at this wavelength. The heating of the sample was carried out with an increase of 1°C per minute where a measuring data was recorded every 1°C.

The sample preparation and recording of the absorbance spectrum of the calf thymus DNA was performing analogously to the Plasmid- DNA. The melting point determination was carried out in the 'cyclic' measurement mode with a start temperature of 70°C and a end temperature of 95°C. Whereby in the range from 70°C to 80°C the optical measurement was performed every 0.2°C; in range from 80°C to 90°C every 0.1°C and from 90°C to 95°C again every 0.2°C. The temperature was controlled with an accuracy of 0.1°C. During the entire analysis the exact temperature was monitored by means of the measuring sensor in the cell.

Results and discussion

The melting process of the DNA-samples could be shown very well in both measurement modes (Figure 4). Because of the base stacking the structure modification of the DNA is performed within a narrow temperature interval. At a defined temperature the double helical structure will be broken down very fast where the absorbance will increase rapidly. In 'cyclic' measurement mode it is possible to set different intervals of several temperature ranges. The melting curve of the thymus DNA could be shown more accurately by setting smaller measurement points in the range where absorbance increases (Figure 6).

The melting points of both DNA samples are derived from the peak maximum of first deviation of the melting curves. The absorbance increase of the bacterial DNA has taken at lower temperatures compared to the thymus DNA. The melting temperature of plasmid DNA and calf thymus DNA are determined at 46.3°C (Figure 5) and at 85.7°C (Figure 7), respectively.

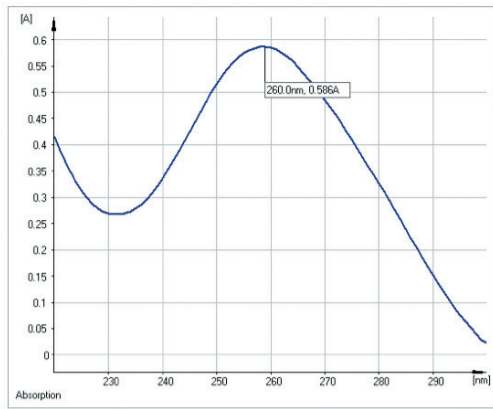


Figure 3: Spectrum of Plasmid - DNA

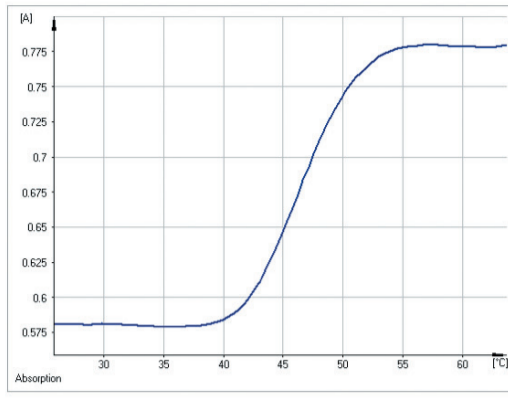


Figure 4: Melting curve of Plasmid - DNA

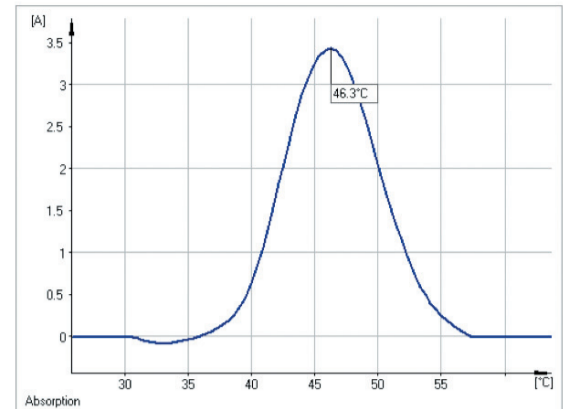


Figure 5: Melting point of Plasmid - DNA

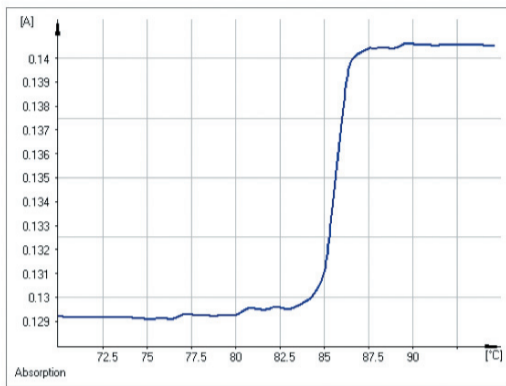


Figure 6: Melting curve of thymus - DNA

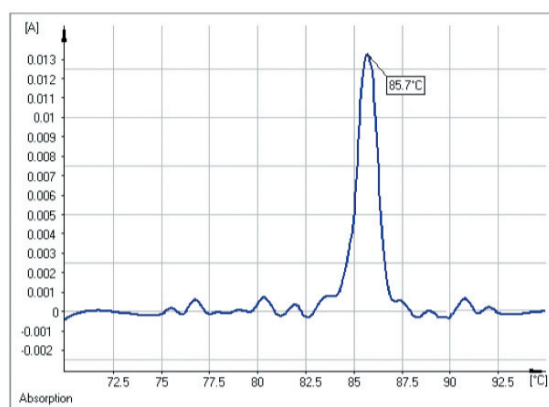


Figure 7: Melting point of thymus - DNA

Native DNA melts in general at high temperatures often above 85°C like the melting point of human DNA which is at 86°C. The melting point of synthetic DNA can be estimated according to the base composition and the chain length.

The lower melting point of the Plasmid DNA is a result of the short chain length of the molecule having 48.5 base pairs (bp). The thymus DNA is composed more complex having a molecular weight of 13 kilobase pairs (kbp). Therefore the melting point of the thymus DNA is correspondingly higher.

Furthermore the melting point of nucleic acids depends on other factors like the chemical composition of formed hybrids, the salt concentration of the buffer solution as well as the specific reagents that are given to destabilise the DNA. Besides the higher proportions of mismatches of the base pairs the lower the melting point will be.

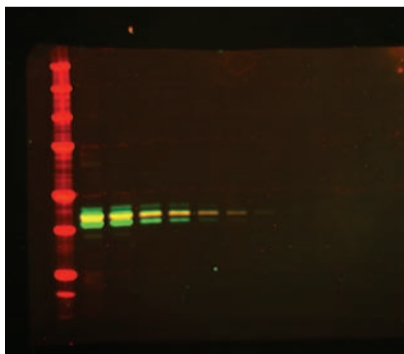
Summary

Nucleic acids store life's blueprint in their special molecular structure. The hyperchromic effect, which is caused by a temperature increase separating the DNA double-strands, allows the DNA melting point to be determined by UV spectroscopy. The SPECORD® PLUS, in combination with peltier tempered accessories, allows the highly precise determination of melting points. Depending on the DNA material and the applicative requirements, temperatures can be programmed over a wide range and in different measurement modes. Use of an ultra-microcell makes it possible to work with microlitre sample volumes. A temperature sensor in the cell exactly monitors the sample temperature and makes sure that it is always equal to the programmed temperature and heating delays do not occur. With this configuration a temperature accuracy of $\pm 0.1^\circ\text{C}$ is attained.

References

- [1] Peter Yakovchuk, Ekaterina Protozanova and Maxim D. Frank-Kamenetskii. Base-stacking and base-pairing contributions into thermal stability of the DNA double helix. *Nucleic Acids Research* 2006
- [2] Allgemeine Mikrobiologie, Georg Fuchs, Hans Günther Schlegel, 8. Auflage, Thieme Verlag
- [3] Genetik, Jochen Graw, 4. Auflage, Springer Verlag Berlin Heidelberg, 2006
- [4] SPECORD® PLUS manual, Analytik Jena
- [5] SPECORD® PLUS accessory manual, Analytik Jena

New IR Imaging Application



Syngene announced that its new range of G:BOX Chemi advanced multi-application image analysers can be used for imaging with infra red (IR) LI-COR IRDye® dyes, making it easier to detect and quantify different types of proteins on multiplex Western blots.

The G:BOX Chemi systems, when fitted with a combination of recommended lighting and specific Syngene filters can be used for imaging LI-COR dyes, IRDye® 680 (Epi Red Multiplex LED lighting module and Syngene 705M filter) and IRDye® 800 (Epi LED IR 740 lighting module and Syngene LY800 filter). The GeneSys software in the G:BOX Chemi automatically selects the right lighting and filters for whichever IR dye or other fluorescent dyes scientists inform the system is on the blot. The software then captures one perfect image of all the different dyes, to ensure imaging fluorescent multiplex Westerns is quick and simple.

Laura Sullivan, Syngene's Divisional Manager, explained: "Scientists want to use fluorescence to visualise proteins on Western blots because they can increase throughput by using the same blot to detect different proteins, something they can't do using chemiluminescent-based blots. Additionally, it can often be difficult to accurately quantify proteins as some fluorescent dyes have overlapping spectra and membranes can auto-fluoresce, which interferes with detecting low abundance proteins. Using IR dyes can sometimes solve these problems."

"Detecting IR dyes has proved difficult using CCD-based systems and so we are excited to have found filters and lighting combinations to allow our G:BOX Chemi to visualise multiplex Westerns of LI-COR IR dyes. This breakthrough means scientists with a G:BOX Chemi now have a sensitive, accurate method for imaging IR labelled proteins without having to buy an expensive laser-based scanner," Laura Sullivan added.