How-to-do-it

DNA Melting Point Laboratory

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The Cell Biology Course at Smith College includes an extensive discussion of the structure of DNA and the processes of DNA replication. We use a specially designed laboratory exercise to illustrate the ease of separation of the component chains of the DNA helix, a basic characteristic of the model that explains transcription and DNA replication (Watson 1976). In the laboratory, students construct a DNA melting curve by observing changes in the viscosity of a DNA solution as temperature is changed. This simple exercise approximates viscosity by timing the flow of the DNA solution through a pipette.

Introduction

Denaturation is the process of breaking the native structure of DNA and can be accomplished by adding acid or alkali to a DNA solution or by increasing the temperature of a DNA solution. We will use a controlled increase in temperature to study DNA denaturation. Thermal denaturation of DNA occurs within a narrow temperature range (Freifelder 1978). The denaturation process causes a helix to coil (randomly oriented single strands) transition that can be followed in a variety of ways. Most commonly the absorbance of a DNA solution at 260 nm is observed as the temperature of the solution is increased. As the temperature increases, more hydrogen bonds are broken and the absorbance increases. More simply, denaturation can be followed by monitoring changes in another physical property of DNA—viscosity. Viscosity is the resistance of a fluid to flow or its stickiness. DNA in solution is a high molecular weight polymer arranged in a stiff and extended conformation that makes the solution highly viscous. Viscosity can be measured by recording the time required for a DNA solution to pass through a small-bore pipette. More viscous solutions will take longer to pass through the pipette than less viscous solutions. Because of the conformation of the DNA molecules, shearing forces such as high-speed stirring or pipetting may cause breakage in the chains.

Molecules of DNA with a high percentage of G:C base pairs are more resistant to thermal denaturation than A:T- rich molecules. Three hydrogen bonds hold G-C base pairs together; only two hold A-T pairs together. Therefore, higher temperatures are required to separate the G-C rich strands than the A-T rich strands. The melting temperature (temperature at which the helix to coil transition is 50% complete) thus indicates the A + T/G + C ratio in the DNA of an organism. For example, the T_m (melting temperature) of the bacterium *Escherichia coli* (strains B, C, K12) is 90.5°C (50.1 mole % G-C); the bacterium *Proteus vulgaris* has a T_m of 85°C (36.5 mole % G-C) [Freifelder 1976]. It may be desirable to provide the class with DNA solutions from various organisms with different mole % G-C content and have them rank the DNA solutions as to richness of the G-C strands.

Even complete denaturation is not necessarily an irreversible phenomenon. If a heated DNA solution is cooled slowly, a single strand can often meet its complementary strand and reform a regular double helical molecule. In our experiment, we demonstrate this phenomenon by slowly cooling the solution of denatured DNA and comparing its viscosity with a solution that has been rapidly cooled.

Materials

Glassware: All the glassware should be baked to destroy nucleases. Human skin is full of nucleases, so caution students not to touch any glassware that will come in contact with the DNA solution, particularly the tips of pipettes. Each team of students will need three test tubes, test tube holders, one 0.1 ml pipette, and a stopwatch.

Waterbaths: A minimum of five waterbaths are needed for this lab. Set the baths at 30°C, 50°C, 70°C, 90°C, and 100°C. Also set up an ice bath.

DNA Solution: Dry DNA from

Salmon testis can be purchased from commercial biochemical supply companies. It is dissolved on acetate buffer pH 5.5 to make an 0.7% solution. After gentle mixing, homogenize gently in a ground-glass homogenizer. This mixture should flow 0.04 ml in less than five minutes at room temperature.

Instructions for Students

The instructions we provide for our students on procedure and directions on handling their data are as follows:

Procedure: Normally viscosity is measured by using a viscometer, but we have simplified the procedure. We will measure the time in seconds that it takes a DNA solution to fall from 0.04 to 0.08 ml in an 0.1 ml pipette. This time will be considered a measure of relative viscosity.

1. Mark three previously baked test tubes with your initials. Place one in the ice bucket. Each team should pour approximately 2 ml of the DNA solution into another tube. Do not pipette the solution. Why not pipette? (Pipetting should be avoided when possible to minimize changes in viscosity due to shearing.)

2. Draw the solution very slowly to minimize shearing into a 0.1 ml pipette to the 0.04 mark. Be careful not to touch the pipette tip—your hands will leave nucleases. Hold the tip of your pipette to the side of the test tube keeping the pipette vertical. Using a stopwatch, measure the length of time in seconds that is required for the solution to fall to the 0.08 mark. Record your results, and note the temperature in your laboratory room.

3. After all the DNA solution has drained from the pipette, equilibrate the solution and pipette (in an empty baked test tube) for five minutes at the next highest temperature.

4. Repeat step 2, recording the temperature of the water bath and time for the flow of DNA solution equilibrated in the water baths of increasing temperature until you reach the boiling water bath.

5. After determining the relative viscosity in the boiling water bath, pour about 1 ml into the cold test tube and quickly resubmerge it into the ice. After ten minutes, let the solution come to room temperature.

6. With the remaining 1 ml, determine the speed of flow in reverse order from hottest temperature to room temperature,

7. Determine the speed of flow for the quickly cooled DNA that is at room temperature.

Results: Plot your results as relative viscosity versus temperature for both increasing and decreasing temperatures on the same graph. Can you estimate the T_m for Salmon testis DNA? Is the T_m the same for the denaturation and renaturation curves? Explain. Describe what happens to the DNA solution that was cooled quickly.

This quick and dramatic exercise generates many thoughtful questions from the students. Usually the denaturation curve is above the renaturation curve and the melting temperatures are different. Many students will perceive that renaturation is not complete and comment on the fact that fewer bonds mean that the T_m for renaturation will be lower. Be prepared to answer questions about the effects of pipetting and shearing force as well as about evaporation causing changes in viscosity.

Acknowledgment—We would like to dedicate this article to the memory of our colleague, George de Villafranca, who initially suggested the idea for this laboratory exercise and whose interest in undergraduate instruction continues to inspire us all.

References

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