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Experiment 17 – Viscosity & Secondary Structure of DNA

Discussion

In 1953, James Watson and Francis Crick proposed a three–dimensional structure of DNA that is a cornerstone in the history of biochemistry and molecular biology. The double helix they proposed for the secondary structure of DNA gained immediate acceptance, partly because it explained all know facts about DNA, and partly because it provided a beautiful model for DNA replication.

In the DNA double helix, two polynucleotide chains run in opposite directions. This means that at each end of the double helix, there is one 5'–OH and one 3'–OH terminal. The sugar phosphate backbone is on the outside, and the bases point inward. The bases are paired so that for each adenine (A) on one chain, a thymine (T) is aligned opposite it on the other chain. Each cytosine (C) on one chain has a guanine (G) aligned with it on the other chain. The AT and GC base pairs form hydrogen bonds with each other. The AT pair has two hydrogen bonds; the GC pair has three hydrogen bonds.

Most of the DNA in nature has the double helical secondary structure. The hydrogen bonds between the base pairs provide the stability of the double helix. Under certain conditions, the hydrogen bonds are broken. During the replication process itself, this happens, and parts of the double helix unfold. Under separate conditions, the whole molecule unfolds, becomes single stranded, and assumes a random coil conformation. This can happen in denaturation processes aided by heat, extreme acidic or basic conditions, etc. Such a transformation is often referred to as helix–to–coil transition. There are a number of techniques that can monitor such a transition. One of the most sensitive is the measurement of viscosity of DNA solutions.

Viscosity is the resistance to flow of a liquid. Honey has a high viscosity and gasoline a low viscosity at room temperature. In a liquid flow, the molecules must slide past each other. The resistance to flow comes from the interaction between the molecules as they slide past each other. The stronger this interaction, the greater the resistance and the higher the viscosity.

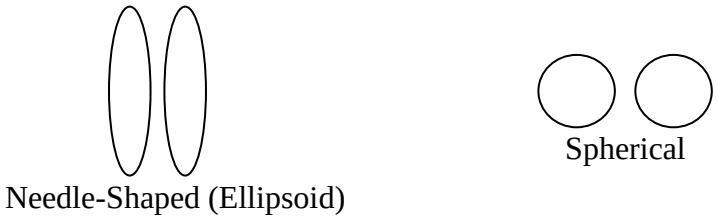

Figure 1: *Surface area of interaction between molecules of different shapes*

Even more than the nature of the intermolecular interaction, the size and the shape of the molecules influence their viscosity. A large molecule has greater surface over which it interacts with other molecules than does a small molecule. Therefore, its viscosity is greater than that of a small molecule. If two molecules have the same size and the same interaction forces but have different shapes, their viscosity will be different. For example, needle–shaped molecules,

when aligned parallel by the flow of liquid, have greater surfaces of interaction than spherical molecules of the same molecular weight (see **Figure 1**). The needle–shaped molecule will have a higher viscosity than the spherical molecule. The DNA double helix is a rigid structure held together by hydrogen bonds. Its long axis along the helix exceeds by far its short axis perpendicular to it. Thus, the DNA double helix has large surface area and consequently high viscosity. When the hydrogen bonds are broken and the DNA molecule becomes single stranded, it assumes a random coil shape, which has much lower surface area and lower viscosity. Thus a helix–to–coil transition is accompanied by a drop in viscosity.

In practice, we can measure viscosity by the efflux time of a liquid in a viscometer (your instructor will demonstrate in lecture). *PLEASE BE CAREFUL WHEN USING THESE VISCOMETERS; THEY ARE VERY DELICATE AND EXPENSIVE.* The capillary viscometer is made of two bulbs connected by a tube in which the liquid must flow through a capillary tube. The capillary tube provides a laminary flow, in which concentric layers of the liquid slide past each other. Originally, the liquid is placed in the storage bulb. By applying suction above the capillary, the liquid is sucked up past the upper calibration mark. With a stopwatch in hand, the suction is released, and the liquid is allowed to flow under the force of gravity. The timing starts when the meniscus of the liquid hits the upper calibration mark. The timing ends when the meniscus of the liquid hits the lower calibration mark of the viscometer. The time elapsed between these two marks is the efflux time.

With dilute solutions such as the DNA in this experiment, the viscosity of the solution is compared to the viscosity of the solvent. The efflux time of the solvent (aqueous buffer) is t_o, and that of the solutions is t_s. The relative viscosity of the solution is:

$$\eta_{rel} = t_s/t_o$$

The viscosity of a solution also depends on the concentration; the higher the concentration, the higher the viscosity. In order to make the measurement independent of concentration, a new viscometric parameter is used, which is called intrinsic viscosity, [η]. This number is calculated by:

$$[\eta] = (\log \eta_{rel}) / c$$

which is almost a constant for a particular solute (DNA in our case) in very dilute solutions. Please note that c represent the concentration of the DNA solution; log represents the logarithm mathematical function as discussed earlier in lecture.

In this experiment, we follow the change in the viscosity of a DNA solution when we change the pH of the solution from the very acidic (pH 2.0) to very basic (pH 12.0). At extreme pH values, we expect that the hydrogen bonds will break, and the double helix will become single–stranded random coils. A change in the viscosity will tell at what pH this happens. We shall also determine whether two acid–denatured single stranded DNA molecules can refold themselves into a double helix when we neutralize the denaturing acid.

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Procedure

Because of the limited number and cost of the viscometers, students may work in groups of 5 – 6. Each group will need two viscometers.

Viscosity of DNA Solutions

1. To 3 mL of a buffer solution, add 1 drop of 1.0 M HCl using a Pasteur pipet. Measure its pH with a universal pH paper. If the pH is above 2.5, add another drop of 1M HCl. Measure the pH again. Record the pH on your data sheet (as #1).

2. Clamp one clean and dry viscometer on a stand. Pipet 3 mL of your acidified buffer solution into bulb A of your viscometer. Using a suction bulb of a Spectroline pipet filler, raise the level of the liquid in the viscometer above the upper calibration mark. Release the suction by removing the suction bulb and time the efflux time between the two calibration marks. Record this as t_o on your data sheet (as #2). Remove all the liquid from your viscometer by pouring the liquid out from the wide arm. Then apply pressure with the suction bulb on the capillary arm of the viscometer and blow out (NOT WITH YOUR MOUTH!) any remaining liquid into the storage bulb; pour out this residual liquid.

3. Take 3 mL of the prepared DNA solution. Add the same amount of 1 M HCl as above (1 or 2 drops). Mix it thoroughly by shaking the solution. Test the pH of the solution with a universal pH paper and record the pH (as #3) and the DNA concentration of the prepared solution on your data sheet (as #4).

4. Pour the acidified DNA solution into the wide arm of your viscometer. Using a suction bulb, raise the level of your liquid above the upper calibration mark. Release the suction by removing the suction bulb and measure and record the efflux time of the acidified DNA solution on your data sheet (as #5).

5. Add the same amount (1 or 2 drops) as above of neutralizing 1M NaOH solution to the liquid in the wide arm of your viscometer. With the suction bulb on the capillary arm, blow a few air bubbles through the solution to mix the ingredients. Repeat the measurement of the efflux time, and record it on your data sheet (as #6). For the next 100 min or so, repeat the measurement of the efflux time every 20 min, and record the results on your data sheet (as #7 – #11).

pH Dependence of the Viscosity of DNA Solutions

6. While the efflux time measurements in viscometer no.1 are repeated every 20 min, another dry and clean viscometer will be used for establishing the pH dependence of the viscosity of DNA solutions. First, measure the pH of the buffer solution with a universal pH paper. Record it on your data sheet (as #12). Second, transfer 3 mL of the buffer into viscometer no. 2 and measure its efflux time on your data sheet (as #13). Empty the viscometer as instructed in step 2 above. Test the pH of the DNA solution with a universal pH paper (as #14) and transfer 3 mL into the viscometer. Measure its efflux time, and record it on your data sheet (as #15). Empty your viscometer.

7. Repeat the procedure described in step 6, but this time, with the aid of a Pasteur pipet, add one drop of 0.1 M HCl to the 3 mL buffer solution as well as to the 3 mL DNA solution. Measure the pH and the efflux times of both buffer and DNA solutions and record them (as #16 – #19) on your data sheet. *Make sure that you carefully empty the viscometer after each viscosity measurement.*

8. Repeat the procedure described in step 6, but this time, add one drop of 0.1 M NaOH solution to both the 3 mL buffer and 3 mL DNA solutions. Measure their pH and efflux time and record them on your data sheet (as #20 – #23).

9. Repeat the procedure described in step 6, but this time, add 2 drops of 1 M NaOH to both buffer and DNA solutions (3 mL of each solution). Measure and record their pH and efflux times on your data sheet (as #24 – #27).

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Data and Calculations for Experiment 17

Viscosity of DNA solutions

(1) pH of acidified buffer _____
(2) Efflux time of acidified buffer (t_0) _____ sec
(3) pH of acidified DNA solution _____
(4) Concentration of DNA solution _____
(5) Efflux time of acidified DNA solution _____ sec
(6) Efflux time of neutralized DNA solution at time of neutralization _____ sec
(7) 20 min. later _____ sec
(8) 40 min. later _____ sec
(9) 60 min. later _____ sec
(10) 80 min. later _____ sec
(11) 100 min. later _____ sec

pH dependence of the viscosity of DNA solutions

(12) pH of neutral buffer _____
(13) Efflux time of neutral buffer _____ sec
(14) pH of DNA solution in neutral buffer _____
(15) Efflux time of DNA in neutral buffer _____ sec

After addition of 1 drop of 0.1 M HCl

(16) pH of buffer _____
(17) Efflux time of buffer _____ sec
(18) pH of DNA solution _____
(19) Efflux time of DNA solution _____ sec

After addition of 1 drop of 0.1 M NaOH

(20) pH of buffer _____
(21) Efflux time of buffer _____ sec
(22) pH of DNA solution _____
(23) Efflux time of DNA solution _____ sec

After addition of 2 drops of 1 M NaOH

(24) pH of buffer _____
(25) Efflux time of buffer _____ sec
(26) pH of DNA solution _____
(27) Efflux time of DNA solution _____ sec

Tabulate your data on the pH dependence of relative viscosity

pH	η_{rel}
(3) _____	(5) / (2) _____
(14) _____	(15) / (13) _____
(18) _____	(19) / (17) _____
(22) _____	(23) / (21) _____
(26) _____	(27) / (25) _____

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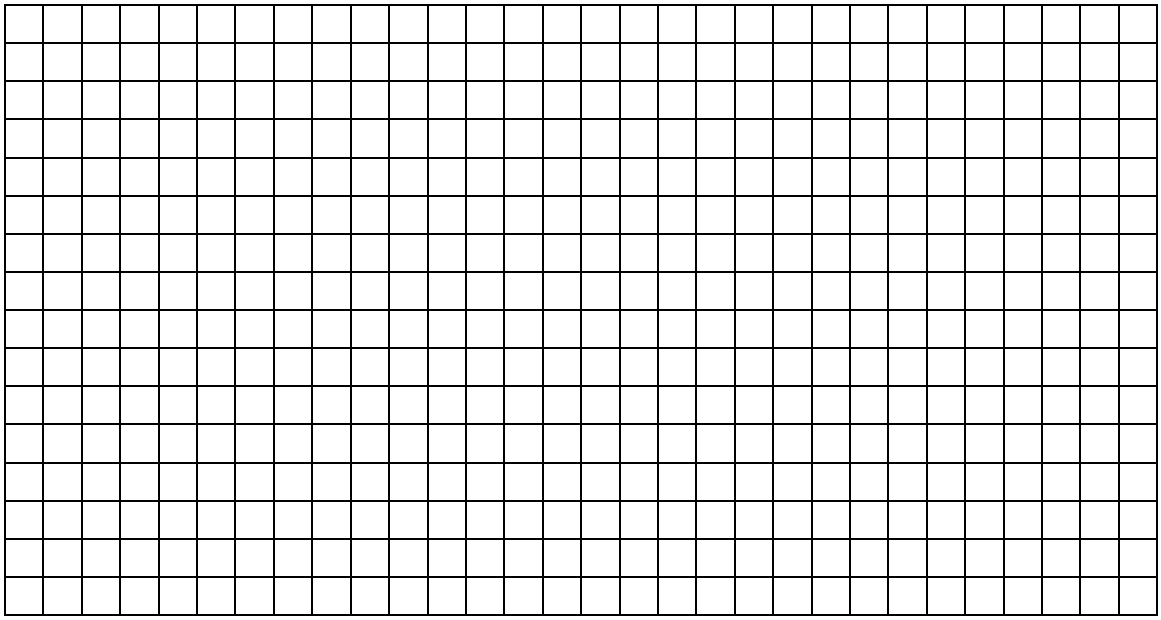
Questions

1. Plot your tabulated data: relative viscosity on the y–axis, and pH on the x–axis.



2. At what pH values did you observe helix-to-coil transitions?

3. Now plot your data on the refolding of DNA double helix (5) – (11) using Microsoft Office Excel®. Plot time on the x–axis (i.e., time after neutralization in min.) and the efflux times on the y–axis (in sec.). Make sure to include this graph with your report. See Experiment #18 for directions on using Excel®. Include the best-fitting line for the data points; *please note that this graph is NOT linear.*

4. Was there any indication that, upon neutralization of the denaturing acid, the DNA did refold into a double helix? Explain.

5. Compare the efflux time of the neutral DNA (15) to that of the denatured DNA 100 min. after neutralization (11). What does the difference between these two efflux times tell you regarding the refolding process?

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6. Calculate the intrinsic viscosity of your DNA at:

   a. Neutral pH = 2.3 × {log [(15) / (13)]} / (4) =

   b. Acidic pH = 2.3 × {log [(5) / (2)]} / (4) =

   c. Basic pH = 2.3 × {log [(27) / (25)]} / (4) =

   d. Neutral pH 100 min. after neutralization = 2.3 × {log [(11) / (13)]} / (4) =

7. A high intrinsic viscosity implies a double helix; a low intrinsic viscosity means a random coil. What do you think is the shape of the DNA after acid denaturation and subsequent neutralization? (See 6d above.) Explain your answer.