"Centrifugal Separation of dsDNA from an ssDNA Oligomer Mixture"

Taiquitha T. Robins

Senior Biology Major

Department of Biology

Dr. Francis Tuluri, Advisor

College of Science, Engineering, and Technology

Jackson State University

Jackson, MS 39217

Research Experience for Undergraduates

Liquid Crystal Materials Research Center

Professor Noel A. Clark, Director

Department of Physics

University of Colorado Boulder

Boulder, CO 80309-390

Project Timelines:

June 5, 2007-August 9, 2007

June 1, 2008-August 8, 2008

Research Outline

1. **Background in DNA**
2. **Background in Liquid Crystals**
3. **Experimental Design**
    a. **Purpose**
    b. **Materials and Methods**
    c. **Results**
    d. **Conclusion**
4. **References**
5. **Acknowledgements**

1. Background in DNA

Deoxyribonucleic Acid (DNA) is the very basic molecule of all living systems. It is the molecule that determines the function of a protein, and the one that provides the instructions carried out for cells and human systems to function. DNA structure is very intriguing. Two strands of DNA join together to form a helix within the cellular nucleus. This helix has many functions. One such function includes pre-reproductive alignment.

The main feature of DNA that makes it particularly interesting for the purposes of this experiment and other biophysics studies is the stacking ability of the helices. DNA is composed of nucleotides. The nucleotides combine together with one another because their tails are hydrophobic and prefer to be away from water.

A DNA helix has a width of 22-26 Ångströms, which is approximated to 2 nm. The helix is bound together by a series of ester bonds on the backbone and hydrogen bonds connecting the horizontal plane of bases. The helix of DNA is polar possessing a negative 5' end, and an alternate 3' end. As the figure to the left depicts, 1 turn of the DNA helix is equivalent to 3.4 nanometers and approximately 10 base pairs. The DNA strands that are being used in this project are 12 bases (base pairs) in length. This project focuses on DNA oligomers. DNA oligomers are those strands of DNA consisting of 10-100 bases (base pairs).

2. Background in Liquid Crystals

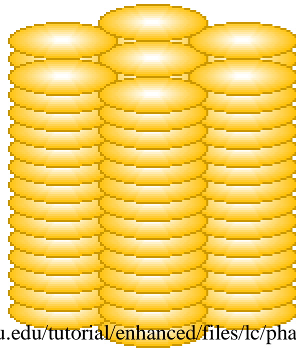
Liquid crystals (LCs) are molecular structures with the stability characteristic of solids, and the fluidic movement of liquids. They are found in two main categories, thermotropic and lyotropic. Thermotropic liquid crystals are solely dependent upon the temperature of the material in which they are known to form. Lyotropic liquid crystals are dependent upon, not only the temperature of the system, but also the concentration of the same. The main difference in the two categories of LCs is that in the lyotropic category, there exists the need for some solvent, which is usually water.

The historical discoveries of liquid crystals are often dated back to 1988, when an Australian botanist named Friedrich Reinitzer performed some experiments with cholesterol. Although, there are some reports which date the discovery of lyotropic liquid crystals in particular to 1850, Reinitzer work is given the most credit. His main contribution was discovering the two boiling points of cholesteryl benzoate. His work was performed in concert with and continued by Otto Lehmann and von Zepharovich. The work performed by these distinguished gentlemen paved the way for much of the liquid crystal work that has been performed since.

There are several known phases of liquid crystals. However, only some of those phases are actually visible in DNA. Therefore, these are the only phases which will be discussed in this paper.

The nematic phase of liquid crystals is the most commonly observed phase in liquid crystals. This phase consists of an orientational arrangement of the liquid crystal complex, but it does not exhibit positional ordering, as shown in the figure to the right. http://en.wikipedia.org/wiki/Liquid_crystals

Columnar liquid crystals, however, exhibit a phase that has orientational and positional ordering. The columnar phase is typically seen in “discotic” liquid crystals and is the transition phase from discotic nematic liquid crystals. There are, however, some reports which indicate that it is not necessary for the columnar phase to possess “disc-like” shapes, and thus the phase can simply be referred to as columnar, rather than discotic columnar. In either case, the phase is characterized by the stacking of liquid crystal domains into packed columns which are ordered in vertical and horizontal directions.



http://plc.cwru.edu/tutorial/enhanced/files/lc/phase/phase.htm

Based on work performed by Giuliano Zanchetta, at concentrations of 600 mg/ml, one will first observe the transition from isotropic to nematic. Then, with increased concentration, one will continue to notice the nematic phase transition into the columnar phase. See the diagram located below.

This diagram is in direct relationship with the work which this paper seeks to describe. And will be explained in further detail later in the paper.

3. Experimental Design

This section of the paper will cover the actual experimental process. From beginning to end, it will detail the process by which we attempted centrifugation of samples in order to promote the separation of single-stranded DNA (ssDNA) from double-stranded DNA (dsDNA). It will cover the purpose of this work, the materials and methods used the results, conclusion, and future works.

3a. Purpose

It is believed that the segregation of complementary and non-complementary DNA strands can be achieved using a physical property of matter called liquid crystals. Complementary strands of DNA exhibit this phase of matter. LC phases should be observable through creation of a concentrated solution. After the formation of this phase, centrifugation should provide for the segregation of these DNA strand types. Complementary and self-complementary DNA have greater tendencies to form liquid crystal phases than non-complementary. Through centrifugation, we should be able to separate an oligomer mixture of ssDNA strands that are complementary to one another. It is with this initial investigation technique that we will be able to further explore the possibilities of the separation of complementary DNA within a DNA “soup”. Separation of the complementary strands of DNA from a “soup” containing numerous non-complementary strands will be the final step of the centrifugation stage of this project.

3b. Materials and Methods

DNA Synthesis:

With the assistance of Mark Moran, of the Chemistry Department at CU Boulder, we are able to synthesis DNA and create single strands of DNA consisting of specific bases. Such synthesis has resulted in the creation of the following 12mer sequences, which are complementary to one another, but are not self-complementary.

5'-CCTCAAAACTCC-3' 3'-GGAGTTTTGAGG-5'

Some complications that arose from the synthesis of DNA were that upon making the aqueous solution, there were noticed crystals in the solution. It was determined that the crystals were the result of impurities in the sample. This problem was resolved by simply desalting the solutions prior to drying and creating the aqueous solutions which were to be viewed using optical polarizing microscopy.

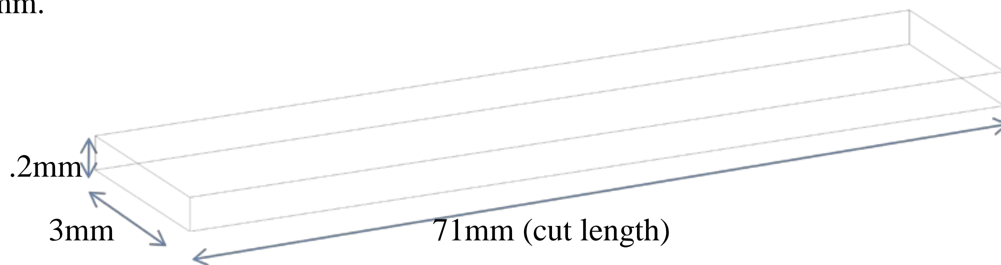
Creation of Concentrated DNA Solutions:

Once the DNA was created, we were able to observe the liquid crystal characteristics of the DNA utilizing optical polarizing microscopy. The samples were created by first weighing the lyophilized DNA using the most accurate scale available to the center. Once the DNA was weighed, a specific amount of Millipore "ultra pure" water was added to the sample. The concentration of the sample was a direct result of how much water was added, rather than the initial weight of the DNA. Thus higher concentrations were achieved by the addition of very small amounts of water. Lower concentrations were thus created using greater amounts of water. No amount of DNA weighed was over 40 mg, and thus no amount of water added was

over 200 µL. The main concentration which exhibited liquid crystal phases of nematic or columnar were at 1000mg/ml created concentrations. The actual concentration is directly dependent upon the density of the DNA which occupied some volume in the solution. Thus, the details pertaining to the solutions can be found in the “results” section of this paper.

Rectangular Capillaries:

Rectangular capillaries having the following dimensions were created from original lengths of 100mm.



These capillaries were filled to about 5-10mm with the created sample and then sealed on both ends with 5-minute Epoxy to prevent evaporation. Evaporation of the sample would increase the concentration and was not ideal because it would violate the concentration which was known to exhibit the nematic or columnar phases. However, it should be noted that making the epoxy such that it would not harden before the sample was loaded into the capillary involved approximately 2-3 minutes of elapsed time which the capillaries were not sealed.

Capillary Holders and Centrifuge Tubes:

Holders were created in order to support the very fragile glass rectangular capillaries. These holders were constructed of a polymer which is very stable and allows for centrifugation at high speeds without disruption of the holders beyond a few cracks in the structure. Two types

of holder were created. There were also two holders of each of the following constructions. One holder consisted of an inner metal structure which aided in guidance and stability of the capillaries. Another holder had the inner polymer carved out so as not to contain the metallic inner piece. The holder not consisting of the metal inner sustained very well through several centrifuge trials. Each set of holders consisted of two main structures which were held together by stainless steel screws. Use of other screw resulted in rusting of the screws when we used water to stabilize the system within the centrifuge tubes during centrifugation. Before centrifugation, the tubes were weighed in their entirety before placement into the centrifuge. Weighing the mini-systems prevented an unbalanced relationship within the centrifuge rotor. Unbalancing the rotor can result in great damage to the centrifuge. The centrifuge tubes containing the holders were filled with water to reduce stress on the capillaries and add some equilibrium to the system in hopes of reducing the breakage of the capillaries. This is still a work in progress.

RC5C Ultra-Centrifuge:

This system was used to spin the capillaries at desirable and settable speeds. The capillaries were initially centrifuged at 20,000 RPM. This speed was determined to be too high for the capillaries, as the force being exerted on them while in the "fixed angle" rotor was over 40,000 g. The next speed tested was 15,000 RPM, and this speed exerted approximately 22,000g. It was also detrimental to the capillaries and resulted in cracking and breaking of the capillaries. The safest speed used was 10,000 RPM with an exerted force of approximately 11,000 g. Capillaries were not centrifuged for more than 24 hours consecutively so as not to damage the ultra centrifuge. Previous centrifugation of

approximately 72 hours over the weekend resulted in damage to the brushes of the centrifuge. The brushes were replaced before any damage to the motor could occur.

3c. Results

1:1	1:4	1:9	1:10
Readily forms liquid crystals regardless of the amount of DNA of either strand added to the solution	**A= 20.15 mg B=5.35 mg 25.23 µL H_2O**	**A= 28.38 mg B=3.57 mg 31.95 µL H_2O Less crowded Batonetts and hexagonal liquid crystals present/visible**	**A= 20.11 mg B= 2.03 mg 24.60 µL H_2O => (900mg/ml) LCs not visible B= +2.50 mg = 1:4 solution (1000 mg/ml) LCs now visible, but highly crowded**

At high concentrations (above 1000 mg/ml) the solution becomes "sticky" and very difficult to handle. It is especially difficult to load the sample into the capillaries at such high concentrations. Thus, it was decided that the safe concentration was 1000 mg/ml, created. The actual concentration for a 1000 mg/ml sample is around 650 mg/ml when calculated based on the following data. It should be noted that the actual concentration is dependent upon the total amount of DNA weighed in addition to the amount of water added with respect to the density of DNA. The "real" concentration of the 1:9 sample is thus calculated using the following equation:

Weight of DNA(mg) / [(weight of DNA(mg)/density of DNA) + amount of water added (µL)]

31.95 / (31.95/1800) + .03195= 642.86 mg/ml

Sample 2A: after 40 hours total centrifugation time

Magnification= 10x

Centrifuge Data:

10,000 RPM

30˚C

It appears, in the above picture that the centrifugation process has worked in this particular sample. However, we have not successfully replicated the process by which this result occurred. It is also not sure whether this result is real due to the fact that there are two menisci. There is still more work to be done to produce desirable and reproducible results.

3d. Conclusion

The work performed in this project leads to some conclusions that separating the dsDNA from a ssDNA oligomer mixture is achievable. Perfected centrifugation is a matter of improvement of the technique of sealing the capillaries and preventing dilution of the sample when water enters the holders and comes into contact with the capillaries. Once these matters are corrected and improved, it is then believed that this process will work smoothly and the future works described below can be accomplished.

Once the technique of centrifuging is accurately performed, the sample can then be tested using mass spectroscopy and other analysis methods to determine whether or not there are single strands mixed with the birefringent double strands or not. Other future projects would

include creating a “soup” of ssDNA strands that are non-complementary to one another containing only two strands which are the complement to each other. This project is a smaller component to a larger work that seeks to prove the function of liquid crystals in the role of DNA stacking in an evolutionary aspect.

Works Cited

www.wikipedia.com (search: “DNA”; “Liquid Crystals”)

Alberts, Bruce; Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walters (2002). *Molecular Biology of the Cell; Fourth Edition*. New York and London: Garland Science.

Pollard, Thomas D.; Earnshaw, William C. Cell Biology.

http://plc.cwru.edu/tutorial/enhanced/files/lc/phase/phase.htm

http://ds9.ssl.berkeley.edu/LWS_GEMS/2/part.htm

http://bly.colorado.edu/lcphysics/lcintro.html

Collings, Peter J.; Hird, Michael (2004). Introduction to Liquid Crystals: Chemistry and Physics. London & New York: Taylor and Francis Group

Zanchetta, Giuliano. Liquid Crystalline Phases in Oligonucleotide Solutions. PhD Thesis. AY 2006-2007

Acknowledgements

"This work is supported in part by the National Science Foundation, Division of Materials Research award #DMR0213918" & "In part by the Minority Access to Research Careers/Undergraduate Student Training in Academic Research (MARC/*USTAR) Program (NIH-MARC 5 T34 GM007672-27)"

- Giuliano Zanchetta
- Chenhui Zhu
- Maggie Hoffman
- Mark Moran
- Arthur
- Noel Clark
- Joe MacLennan
- Christine Morrow
- Dr. Francis Tuluri (JSU)
- The Entire Liquid Crystal Group @ CU Boulder
- REU Program Directors
    - Dan & Deborah
- MARC Program
- Dr. Jacqueline Stevens, JSU
- EVERYONE!!!!

In memory of:

Mrs. Shirley J. Collins-Robins