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An investigation into Ca-DNA conformation as a function of relative humidity

Megan Schwenker Smith
The University of Toledo

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A Dissertation

entitled

An Investigation into Ca-DNA Conformation as a

Function of Relative Humidity

by

Megan Schwenker Smith

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the Doctor of Philosophy Degree in Physics

Dr. Scott Lee, Ph.D., Committee Chair

Dr. Patricia Komuniecki, Dean
College of Graduate Studies

The University of Toledo
August 2010
An Abstract of

An Investigation into CaDNA Conformation as a Function of Relative Humidity.

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Megan Schwenker Smith

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Raman spectroscopy experiments on CaDNA free-standing, highly-ordered wet-spun films containing various concentrations of CaCl₂ show that CaDNA does not adopt the A conformation and reveals a maximum of DNA in the B conformation at 80% relative humidity. Swelling experiments on these same films give information as to the intermolecular spacing between molecules. Finally, a proof of principle measurement of the activation enthalpy of guanosine is also given.
Acknowledgements

Coming from a non-traditional background and attempting a graduate degree in physics involves the help of many people. There were many late nights in the beginning. I have to thank the classmates who were patient enough to stay up with me those nights and explain the same principle about a hundred times until I got it. They include Noel Richardson, Aaron Korostyshevsky, Jonathan Skuza, Jason Stoke and Kyle Walker. I am lucky to know them and grateful to call them my friends.

My advisor, Dr. Lee, is far more than an advisor. He is also a teacher. The clarity he is able to bring to complicated principles is a gift that is not found among many faculty members. There has always been a balance between helping me grow as a student and making me earn my way as a physicist. He has been my advocate and because of Dr. Lee, I have been able to have opportunities to explore different career paths and experience different careers in physics.

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I owe a huge debt of gratitude to my family, especially my sisters. I don’t always give them the credit they deserve but this time there is no denying, I would not have finished my Ph.D. without their help.

Finally, thanks to my husband, Kevin. His love and support has kept me going. He gave up more than he should have to make sure I was able to get my work done. Those sacrifices did not go unnoticed. Thank you, Kevin!
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Chapter 1

I. Introduction

1.A. What is DNA?

The biological significance of deoxyribonucleic acid (DNA) has made this molecule an interesting subject of research since the 1940’s. DNA is the molecule that contains the genes which allow genetic information to be expressed and passed on to following generations. For this reason, DNA is an important molecule to understand.

In 1944 Oswald Avery published a paper stating that DNA was a transformation agent of genetic material. Later, in 1949, Erwin Chargaff proposed that in natural DNA the number of guanine units equals the number of cytosine units and the number of adenine units equals the number of thymine units. These units will be defined later. In 1951 Rosalind Franklin gave a lecture at King’s College stating that DNA can exist in two forms, depending on the relative humidity in the surrounding air. James Watson attended the lecture and, with Francis Crick, attempted to build
their first model of DNA, albeit an inaccurate model. Later, in May of 1952 Rosalind Franklin and Raymond Gosling took the famous photograph using x-ray crystallography which shows the double helical nature of DNA.

In January of 1953 her colleague, Maurice Wilkins, showed Franklin’s photograph to Watson without her consent. It was from this photograph and the discoveries of Avery and Chargaff that Watson and Crick were able to build a correct model of DNA.

The structure of DNA is significant because it proposes an elegant solution to the problem of gene replication. By separating the DNA strands, the individual bases are exposed. The bases bond via the following rules: adenine to thymine and guanine to cytosine. This results in two, identical molecules of DNA coming from the original DNA molecule. This process is called replication and is discussed in detail later. In 1962, Watson, Crick and Wilkins were awarded the Nobel Prize for discovering the structure of DNA.

1.B. DNA Structures

DNA is a long polymeric molecule. It consists of a counterion and a repeat unit, called a nucleotide, made of a phosphate group, deoxyribose and one of four bases: adenine, thymine, guanine and cytosine.
DNA can exist in many geometric forms but the most famous model of DNA is that of Watson and Crick. Since the other geometric forms are similar to the Watson – Crick model it will be discussed first and the other geometries will be discussed later. The Watson - Crick Model of DNA consists of two polymer chains in a double helical form. The DNA consists of a sugar phosphate backbone and on the interior two sets of bases. The polymer chain is made up of a deoxyribose sugar and a phosphate group.

The sugar carbons are numbered 1’ through 5’. The backbone sugars on the DNA run in the opposite direction with respect to the orientation of the oxygen on the sugar ring.

Next, taking just one side of the DNA strand, one can better examine the backbone. Here the DNA sugar phosphate backbone is connected with the four base pairs. Nucleotides are connected through the phosphate group (PO₄) between the 5’ carbon of the deoxyribose sugar ring and the 3’ of the adjacent deoxyribose sugar ring. The phosphate group is negatively charged and has a net charge of 1e⁻ per base or 2e⁻ per base pair. Consequently, all DNA samples contain a counterion for charge neutrality. The counterion is part of the DNA.

The two polymer chains that make up the double helical backbone are held together by the base pairs: adenine (A) with thymine (T), and guanine (G) with cytosine (C) as shown in Figure 1. These A-T and C-G pairs are joined together by weak hydrogen
forces whereas the covalent bonds inside the backbone are stronger. In the case of RNA, thymine is replaced by uracil which binds to adenine. This occurs in transcription which will be discussed later.

Figure 1 – G-C and A-T base pairs with hydrogen bonds. Photo courtesy of U.S. Department of Energy Genome Programs (http://genomics.energy.gov)
1.C. Transcription and DNA Replication

1.C.a. Transcription

Transcription is the first step in producing a protein from a gene (a segment of DNA). The process of transcription varies from one organism to the next. Here, transcription of *E. coli* will be described. Transcription results in the synthesis of a single-stranded RNA molecule. This RNA will then go elsewhere in the cell and be translated into a specific protein. RNA is a single strand composed of a sugar phosphate backbone and bases. The bases are attached to sugar in the same manner as in DNA. However, in the case of RNA, the sugar is ribose instead of deoxyribose. The bases of RNA are guanine, cytosine, adenine and uracil. To make RNA, only one side of the DNA will be read. This side of the DNA is called the template strand. Specific DNA sequences, called promoters, signal a protein where to bind to DNA in order for synthesis to begin. This protein is called RNA polymerase and it makes RNA. The promoter is recognized specifically by the sigma subunit (σ) of the RNA polymerase.

It is believed that the RNA polymerase explores a length of the DNA until the promoter sequence is found. Once this occurs, the DNA is locally unwound and the template strand is exposed for RNA synthesis.
With the template strand exposed, the RNA polymerase catalyzes the insertion of the first ribonucleotide. Subsequent ribonucleotides are inserted and linked together. This is called chain elongation. After a few ribonucleotides have been added, the σ subunit of the RNA polymerase dissociates and elongation continues.\(^5\)

Eventually, the enzyme traverses the entire gene and encounters a termination signal. The RNA is complete and will be translated into protein elsewhere in the cell.

The DNA molecule is constantly changing its geometric shape and interacting with other molecules. Another process that illustrates this is DNA replication.

1.C.b. DNA Replication

All cells divide at some point in their life span. The original cell is known as the parent cell and the resulting two cells are called daughter cells. During cell division everything within the parent cell must divide to ensure the survival of the daughter cells. Of most importance for cell survival is the accurate replication of the cellular genome. The process of replicating the cellular genome is called DNA replication.

DNA begins replication when an enzyme called helicase cleaves the DNA strand apart into two single strands. Single strand DNA-binding (SSB) proteins stabilize the single stands by preventing the two strands from recombining.
One strand is called the leading strand and the other is called the lagging strand. Polymerase III, the enzyme that builds the complement strands by inserting the complementary nucleotides, binds to the leading and lagging strands; however, in the case of the leading strand, Polymerase III immediately synthesizes a new strand on the exposed DNA template.

The reason the leading strand is immediately synthesized is the result of the direction of the sugar in the DNA backbone. Recall the carbons in the sugar ring are labeled 1’ though 5’. Replication synthesis occurs only in the 5’ to 3’ direction. Since the sugar rings in the DNA backbone strands run in opposite directions, this creates a problem for synthesis of the lagging strand as the sugar carbons are oriented in the unfavorable 3’ to 5’ direction. Synthesis of the lagging strand is more involved as it has to overcome the unfavorable orientation of the sugar rings. This means even more molecular interactions need to occur with DNA! An enzyme called DNA primase lays down RNA that acts as a primer to Polymerase III. Polymerase III is signaled by this RNA, called RNA primer, to bind to the DNA at that location. This occurs near the fork. The attached Polymerase III simply works in the direction moving away from the fork. By moving this way, Polymerase III does move in the favorable 5’ to 3’ direction.

As it moves away from the fork, Polymerase III is only able to replicate a fragment of the DNA as it will quickly catch up to the preceding fragment. These fragments are
known as Okazaki fragments. At the fork, the DNA primase is putting together an RNA primer for the second Okazaki fragment.

The original Polymerase III disassociates and another attaches to make a second Okazaki fragment. This is going on while RNA primer prepares for a third Okazaki fragment to be made. DNA synthesis on lagging strand is discontinuous but efficient.¹

However, the template strand is now made up of both DNA and the RNA that was used as primer of Polymerase III. This RNA primer gets removed by Polymerase I.² The joining of adjacent DNA fragments is done by DNA ligase. This continues until the entire DNA strand has been replicated and results in two, new, identical strands of DNA.²

Notice how many molecules interact with DNA just in replication!

In both processes of replication and transcription DNA must interact with other molecules. DNA’s interactions with other molecules are interesting and biologically significant. It is the purpose of this research to better understand the intermolecular interactions of DNA. Our approach is to examine the intermolecular bonds within free-standing, wet-spun films of DNA. Before we proceed however, it is important to discuss the different geometries of DNA as these geometries can be altered according to physical conditions. Specifically, the transition from the A conformation to the B
conformation will be discussed following a thorough explanation of the structure of DNA.
II. Conformation of DNA

DNA can be in any of the following physical conditions:

1. in a solution of water
2. as a gel with water
3. in a living cell
4. in a crystal (which is usually kept in its mother liquor)
5. in a pulled fiber
6. in a wet-spun highly-oriented and free-standing film.

Each of these physical conditions has advantages and disadvantages for experimental work. Recall that genomic DNA in a living cell is found in chromosomes in which the DNA molecule is in a highly condensed state with many interactions with itself and with the proteins of the histone cores.
The Raman and swelling experiments of this dissertation were performed on wet-spun, highly oriented, and free-standing films. The degree of hydration of these films was controlled by varying the relative humidity to which they were exposed.

2.A. DNA Conformations

Five groups of structures of DNA are known to exist. These include the right-handed A-, B-, C-, and D- forms and the left-handed Z-form. Of these five conformations, only three are of biological importance, the A-, B- and Z- conformations. The C- and D- conformations seem to exist only in the laboratory. For this reason, we will limit our discussion to only the A-, B- and Z- conformations of DNA.

The Z conformation exists in solutions in which the salinity is high. The B conformation of DNA exists in solution and the A conformation of DNA is believed to exist only in highly ordered samples. This will be discussed at length later. In addition, there is evidence which shows that the A and B conformations can be controlled by changing the relative humidity and the identity of the counterion in free standing films. In fact, by controlling these parameters, DNA in the A conformation can be driven into the B conformation; likewise DNA in the B conformation can be driven into the A conformation. A B-Z transition occurs by controlling salt concentration and or the addition of multivalent cations. These conformations are shown in Figures 2-4 which are taken from http://www.google.com/imgres?imgurl=http://upload.wikimedia.org/wikipedia/commons/5/56/ADNA%28ana%29-BDNA%28bna%29-

http://www.google.com/imgres?imgurl=http://upload.wikimedia.org/wikipedia/commons/5/56/ADNA%28ana%29-BDNA%28bna%29-
Figure 2 – The A conformation of DNA.
Figure 3 – The B conformation of DNA, also known as the Watson – Crick Model.
Figure 4 – The Z conformation. Rotation of the axis is in the left-handed direction.

The parameters used to define the conformations of DNA are the helix sense, base pairs per turn, axial rise, helix pitch, base pair tilt, diameter of the helix and the rotation per base pair as shown in Figure 5. The helix sense refers to the rotation of the helix, whether it is right or left handed. The base pairs per turn is the number of base pairs needed in one 360° rotation of the helix. The axial rise refers to the distance between the base pairs. The helix pitch is the length of one full turn of DNA. The base pair tilt is the angle the base pair makes with respect to the helical axis. The
The most common form of DNA is the B form. DNA in the B conformation consists of approximately 10 base pairs per complete rotation of the helix. These base pairs are spaced 3.57 Å apart and tilted -6° with respect to a line perpendicular to the helical axis. The diameter across the helix is about 20 Å. The length of one complete turn or the helix pitch is 35.7 Å. As there are 360° in a complete rotation, then a 36° helical twist exists between each base pair. The helix is right-handed and has a major groove and a minor groove.

The A conformation of DNA is similar to the B conformation; however, there are 11 base pairs per complete rotation of the helix, the base pairs are spaced 2.55 Å apart, and the bases are tilted 20° with respect to the helical axis. Also, the base pairs do not
go through the center of the molecule, but are out toward the edge. Finally, the diameter is slightly larger, measuring 23 Å across.\textsuperscript{4}

Z DNA is dramatically different in that its rotation is left-handed. It is also the narrowest of the three families having only a diameter of 18 Å. Because of this, the bases are tilted at -9\textdegree.

Below is a table displaying the differences in the helical parameters of the A, B and Z DNA conformations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>A-DNA</th>
<th>B-DNA</th>
<th>Z-DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helix Sense</td>
<td>Right</td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>Residue per turn</td>
<td>11</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Axial rise (Å)</td>
<td>2.55</td>
<td>3.6</td>
<td>3.8</td>
</tr>
<tr>
<td>Helix pitch (Å)</td>
<td>28</td>
<td>36</td>
<td>45.6</td>
</tr>
<tr>
<td>Base pair tilt (˚)</td>
<td>20</td>
<td>-6</td>
<td>-9</td>
</tr>
<tr>
<td>Rotation per residue (˚)</td>
<td>33</td>
<td>36</td>
<td>30</td>
</tr>
<tr>
<td>Diameter of helix (Å)</td>
<td>23</td>
<td>20</td>
<td>18</td>
</tr>
</tbody>
</table>

2.B. Factors Affecting DNA Conformation

DNA has the ability to change its conformation, as previously mentioned. Through studying these transitions, factors that affect DNA conformation have been identified. These factors include water activity (the number of water molecules interacting with the DNA molecule per base pair), counterion identity and intermolecular bonding.
Here these shall be explained fully. Note that the DNA in this discussion is in free-standing films.

2.B.a. Water Activity

The ability of DNA to change from the A conformation to the B conformation is known as the A to B transition. This transition is most important in helping determine what factors play a role in conformational changes. First, we explore the role that relative humidity plays in regard to the A to B transition. Rosalind Franklin\textsuperscript{7,8} was the first to show that humidity (or water activity) controlled the conformations of NaDNA (which is defined in section 2.B.b). She and Raymond Gosling\textsuperscript{7,8} found that NaDNA is in the A conformation at 75\% relative humidity and in the B conformation at 92\% relative humidity. She is the person who developed the naming convention for the different conformations. Lindsay and co-workers\textsuperscript{9,10} performed experiments on DNA films containing different counterions and determined that the A to B transition occurs at different relative humidities for different counterions. For NaDNA this transition occurs at 92\% relative humidity, for KDNA and RbDNA this occurs at 90\% relative humidity and for CsDNA this occurs at 80\% relative humidity. These results show that the A to B transition occurs at different water contents.
Lavalle et al.\textsuperscript{11} measured the water content for free standing films containing monovalent counterions as a function of relative humidity. Figure 7 shows their results.

Their water content experiments show, to within the experimental uncertainty, that the water contents at a given relative humidity (rh) of Na-, K-, Rb- and CsDNA are
Because the water content curves are similar for these types of DNA films, this indicates that the species of the monovalent counterion does not affect the amount of the water of hydration. The water of hydration refers to the water molecules that are attached to the DNA molecule and come from the ambient humidities (recall that the films are free-standing). Since Lindsay and co-workers\textsuperscript{9,10} have given the relative humidity at which the A to B transition occurs, Lavalle et al.\textsuperscript{11} were able to determine the water content from ambient humidity at these relative humidities.

Table 2 - Counterion Dependence of the A to B Transition in Calf-Thymus DNA

<table>
<thead>
<tr>
<th>Type of DNA</th>
<th>Percent RH\textsubscript{A-B}</th>
<th>Number of water molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>92</td>
<td>41</td>
</tr>
<tr>
<td>K</td>
<td>90</td>
<td>38</td>
</tr>
<tr>
<td>Rb</td>
<td>90</td>
<td>36</td>
</tr>
<tr>
<td>Cs</td>
<td>80</td>
<td>22</td>
</tr>
</tbody>
</table>

Table 2 – The number of water molecules per base pair at the A to B transition for Na-, K-, Rb- and CsDNA.\textsuperscript{11}

Table 2 shows that the A to B transition occurs at different water contents for the different types of DNA. Since the only difference in the different samples of DNA is the counterion, these results indicate that the counterion is of fundamental importance in determining the conformation of DNA. Later we describe a hypothesis about the role of the counterion in controlling the conformation of DNA.
DNA is solvated with water molecules which form shells of hydration around the DNA. Without water, the DNA molecule will collapse and lose its double helix shape.\textsuperscript{12} This hydration is usually thought of as two shells: primary hydration and secondary hydration. The primary hydration shell consists of water molecules that are strongly attached to the DNA while the secondary hydration shell are the water molecules that are more weakly bound to the DNA. Staticaly, the hydration shells help stabilize the DNA molecule and dynamically they interact with the DNA vibrations in the gigahertz range\textsuperscript{13}. Much research has been done to better understand the structure of the hydration shells and the results are nicely summed up by Tao, Lindsay and Rupprecht\textsuperscript{13} in the following:

“There are about 20 water molecules per nucleotide pair in the primary hydration shell. About 12 of them are bound tightly to DNA. The structure of water in the primary hydration shell is substantially different than that of bulk water and is described by some as an ice-like structure. The secondary hydration shell is less well defined. It is similar to bulk water, but it contains the counterions”\textsuperscript{13}

The significance of the hydration shells in this research is that humidity affects the conformation of the DNA. As already mentioned, Lindsay and co-workers\textsuperscript{9,10} completed Raman experiments on DNA samples with different monovalent counterions which showed that the A to B transition occurs at different relative humidities. The A to B transition occurs at 92\% relative humidity for NaDNA, at 90\% relative humidity for KDNA and RbDNA and finally at 80\% relative humidity CsDNA. At the A to B transition, Lavalle\textsuperscript{14} found that the number of water molecules per base pair is 41 water molecules for NaDNA, 38 water molecules for KDNA, 36 water molecules for RbDNA and 22 water molecules for CsDNA. Lavalle\textsuperscript{14} suggest that because the number of water molecules at the A to B transition
is very different for each DNA sample, then the water content is not the only factor involved in the A to B transition.

The purpose of this research is to further develop the role of the counterion and hydration by examining DNA samples with calcium, a divalent counterion, and varying the relative humidities. First, however, the role of the counterion should be explained further.

2.B.b Counterion Identity

In the case of DNA, the counterions have a positive charge. Of interest in this research are monovalent and divalent counterions. Examples of monovalent counterions are Na, Li, K, Rb, Cs, which have a +1e charge. Since DNA samples typically are prepared with the same counterion throughout, the samples are referred to as Li-, Na-, K-, Rb- and CsDNA. The species of counterion has been shown to influence a transition between the A to the B conformation of DNA, as discussed above. Interestingly, LiDNA never adopts the A conformation. Examples of divalent counterions are Mg$^{2+}$, Ca$^{2+}$, Sr$^{2+}$ and Ba$^{2+}$. Since divalent counterions have a charge of +2e it is expected that only half as many divalent counterions are necessary for neutrality as monovalent counterions. As discussed later, the counterions are believed to be part of the intermolecular bond and they influence the relative humidity of the A to B transition.
Duguid et al.\textsuperscript{17} used Raman spectroscopy on calf-thymus DNA to find the binding sites of divalent counterions and compare the perturbations to DNA secondary and tertiary structures induced by alkaline earth ions (Sr\textsuperscript{2+}, Ba\textsuperscript{2+}, Mg\textsuperscript{2+}, and Ca\textsuperscript{2+}) and transition metal ions (Mn\textsuperscript{2+}, Co\textsuperscript{2+}, Ni\textsuperscript{2+}, Pd\textsuperscript{2+}, Cu\textsuperscript{2+}, and Cd\textsuperscript{2+}).

Dugid et al.\textsuperscript{17} take spectra of DNA containing the divalent counterion and subtract a NaDNA spectrum which results in a difference spectra.

Spectral differences throughout Mg\textsuperscript{2+}, Ca\textsuperscript{2+} and Ba\textsuperscript{2+} occur in the region 1200-1500 cm\textsuperscript{-1}. In BaDNA, MgDNA and CaDNA, the 1489 cm\textsuperscript{-1} mode is shifted to a slightly lower mode. This is what generates the peak near 1480 cm\textsuperscript{-1} and the trough near 1495 cm\textsuperscript{-1} in the difference spectra. Other perturbations occur in the 1250-1450 cm\textsuperscript{-1} and 750-950 cm\textsuperscript{-1} regions with the greatest effect appearing in CaDNA.

In the literature review in Duguid’s paper\textsuperscript{17}, it is summarized that most metal counterions bind preferentially to the bases relative to the phosphates in the following order: Hg\textsuperscript{2+} > Cu\textsuperscript{2+} > Pb\textsuperscript{2+} > Cd\textsuperscript{2+} > Zn\textsuperscript{2+} > Mn\textsuperscript{2+} > Ni\textsuperscript{2+} > Co\textsuperscript{2+} > Fe\textsuperscript{2+} > Ca\textsuperscript{2+} > Mg\textsuperscript{2+}, Ba\textsuperscript{2+}. Those with the strongest base affinity seem to perturb hydrogen bonding between base pairs, thereby destabilizing the B form of DNA and stabilizing alternative structural forms. Interestingly, counterions that have a greater affinity for the phosphates stabilize B DNA. Calcium, magnesium and lead were found to have higher affinities for phosphates. In order to determine whether metal induced perturbations of one band are correlated to another, Duguid et al.\textsuperscript{17} use correlation
plots of perturbations of bands observed at 665, 681, 727, 750, 834, 1092, 1257, 1489 and 1668 cm$^{-1}$. Their correlation plots confirmed the preferential order of metal counterions binding to the bases as found in the literature.

Gueron et al.$^6$ put together a unified theory of the B-Z DNA transition in high and low concentrations of multivalent counterions. Gueron et al.$^6$ evaluated the variation of free energy difference with ionic strength. They determined that a modest shift in the nonelectrostatic free energy difference radically changed the behavior of the B-Z equilibrium in a solution of divalent counterions. If a divalent counterion creates such a radical change in the B-Z transition, than what could the introduction of a divalent counterion do to the A-B transition?

2.B.c. Intermolecular Bonds and Conformational Transitions

Understanding the underlying mechanism of conformational transitions of DNA is an area of intense work. It is believed that intermolecular bonding is critical for such changes in the geometry of the DNA molecule. As already discussed, the geometry of the DNA molecule is changed dramatically by the presence of certain proteins during transcription and replication.

Though our ultimate goal is to obtain a complete understanding of transcription and replication, we first work on understanding the related, but much simpler, conformational changes of DNA. In this work, we examine the A and B
conformations and the role that intermolecular bonding plays with these conformations.

Eyster and Prohofsky\textsuperscript{18} have theorized that the A to B transition is the result of a soft mode transition, meaning that the transition would be driven by a factor internal to the DNA. A soft mode transition occurs when the amplitude of a natural vibration of the system grows so large that the original structure is destabilized and the system is driven into a new geometry. This is called a soft mode transition because the frequency of the molecule goes to zero as the atomic geometry is rearranged. With regards to the A to B transition in DNA, Prohofsky wrote,

\begin{quote}
“The actual mode is a conglomerate motion which has the backbones beating against each other (thereby tilting the bases), coupled with the breathing or swelling of the helix…It looks just like the motion required for moving from one conformation to the other”\textsuperscript{19}
\end{quote}

In other words, the softening of the lowest lying optical mode is what causes the A to B transition. However, this theory is incomplete as it does not describe the role of the counterion in terms of environmental factors that drive the A to B transition. Fortunately, the theory does make a prediction about the behavior of low frequency vibrational modes. The frequency of the soft vibrational mode drops as the structure changes. DNA samples convert from A to B form as the water content increases. This kind of behavior is predicted by varying the dielectric constant. At the A to B transition this mode should go to zero.
Lindsey et al.\textsuperscript{20} studied Eyster and Prohofsky’s prediction and found that, although softening in low-lying Raman bands in NaDNA and LiDNA did occur, the frequency of the mode never went to zero. They found that most of the softening could be accounted for as mass loading of bound water.

Raman experiments\textsuperscript{10, 20-22} have resolved five low frequency vibrational modes at approximately 25, 34, 70, 95 and 115 cm\textsuperscript{-1}. Two of these modes soften while the sample is being hydrated, the 25 and 34 cm\textsuperscript{-1} modes. Of these two modes the 34 cm\textsuperscript{-1} mode is observed in solution while the 25 cm\textsuperscript{-1} mode is not. Since a solution contains isolated molecules, the 34 cm\textsuperscript{-1} mode cannot be the result of an intermolecular bond. It is therefore concluded that the 34, 70, 95 and 115 cm\textsuperscript{-1} modes are intramolecular while the 25 cm\textsuperscript{-1} mode is intermolecular. A comparison of low frequency Raman experiments\textsuperscript{21,22} show that frequency of the 25 cm\textsuperscript{-1} mode changes considerably when a change is made in the three dimensional arrangement of the molecules. This reinforces the assignment of this mode as intermolecular. Further support of this assignment is found in a comparison of the 25 cm\textsuperscript{-1} mode in calf thymus DNA and the dodecamer d(CGCGAATTCGCG)\textsuperscript{21,23}. Dickerson et al.\textsuperscript{24} show by means of x-ray diffraction that there is one strongly interacting phosphate group for every 12 base pairs in the dodecamer.

Rupprecht\textsuperscript{25} noticed that NaDNA samples prepared in bathing solutions with varying NaCl concentrations resulted in films of varying degrees of crystallinity. (A description of these samples will come later.) The sharpest x-ray diffraction patterns
were observed in samples bathed in 0.03 M of NaCl. Lindsay and co-workers\textsuperscript{22} performed atomic emission spectroscopy experiments which showed that such samples contain 1 excess NaCl per every 10 base pairs. They then hypothesized that the 1 NaCl (or rather the Na\textsuperscript{+}) is involved in neutralizing close PO\textsubscript{4}-PO\textsubscript{4} clashes of the neighboring molecules. For this reason, Lindsay et al.\textsuperscript{22} believe the excess Na\textsuperscript{+} (from the NaCl) is an integral part of the intermolecular bond which stabilizes the A conformation of DNA.

Lindsay et al.\textsuperscript{22} also used x-ray crystallography to construct a three dimensional model of crystalline DNA. This model showed that for every ten base pairs there is one near clash between free oxygen atoms on the phosphate groups of neighboring DNA molecules. They suggested that the intermolecular bond that stabilizes the A conformation comes from these phosphate groups which bond to the counterions via ion clusters. Lindsey and co-workers\textsuperscript{21} compared the dodecamer to the calf thymus DNA and noticed that the intermolecular bonding should be about 20\% weaker in the dodecamer. Correspondingly, the frequency of the intermolecular mode should be about 10\% less in the dodecamer. Experiments show that the 25 cm\textsuperscript{-1} mode is 12±4\% less in the dodecamer\textsuperscript{20,21}, further supporting Lindsay et al.\textsuperscript{22}.

Rudd et al.\textsuperscript{26} describes an electrostatic theory which shows that the intermolecular bond is necessary to stabilize the A conformation of DNA. Their theory includes both water of hydration and counterions.
Lattices for DNA aggregates are calculated by finding the lattice with the lowest energy state. They find that by decreasing the interaxial separation (corresponding to lowering the water content of our wet-spun films) the interaction energy between helices strengthen such that the difference in energy per base pair between the A and B form shift to favor the A form. This is shown in Figure 8.

Figure 8 –Red line shows energy per base pair for conglomerates of B DNA and blue line shows energy per base pair for conglomerates of A DNA. Data taken from reference 26.
Calculations without intermolecular interactions (i.e. an isolated DNA molecule) show that the A conformation is never at a lower energy than the B conformation. Therefore, these calculations also find that intermolecular interactions are necessary to stabilize the A conformation.

In order to test the hypothesis that the intermolecular bonds are necessary to stabilize the A conformation of DNA, Lindsay and co-workers\textsuperscript{22} prepared two sets of samples. The only difference between the two sets of samples is that one set were highly crystalline films with many intermolecular bonds between neighboring DNA molecules while the other set were disordered films with relatively few intermolecular bonds. The counterions for these experiments are Na\textsuperscript{+}.

The two sets of samples were cut from the same wet-spun, highly oriented crystalline films. One set of samples had no modifications made to them and were the crystalline samples. The other set of samples were hung from a wire and small amounts of pure water were dropped onto them. This caused the wet-spun films to expand dramatically in the direction perpendicular to the molecular axis and all crystalline order was lost and DNA gel was formed. Great care was taken to ensure that no water dripped off the sample. The gel was then held at room humidity until all the water evaporated. The resulting film had no crystalline order as was confirmed by the lack of any optical birefringence. X-ray diffraction patterns also showed no crystallinity in the samples. However, the amount of NaCl in the amorphous films remained the same as in the pristine crystalline films since no water
dripped off the films while they were in a gel state. Therefore, these two sets of films were identical except for the degree of crystallinity.

Raman experiments were then performed on these two sets of films. The water activity was not controlled by having the films exposed to different relative humidities. Instead the films were submerged in mixtures of ethanol and water. DNA films do not dissolve in ethanol/water mixtures provided the relative amount of ethanol is high enough. Higher amounts of ethanol correspond to lower amounts of water (i.e., lower water activity). Recall that for NaDNA the A conformation is observed below 92% relative humidity; that is, at lower water contents. Consequently, the A conformation will be observed in ethanol/water solutions with lower amounts of water and higher amounts of ethanol. Note that Figure 9 is labeled by the amount of ethanol rather than the amount of water.

Figure 9 shows the presence of the vibrational band at 807 cm\(^{-1}\) (marked by the arrow) in the ordered sample (a) but not in the disordered sample (b). This Raman mode observed at 807 cm\(^{-1}\) is present only when it is in the A conformation. (More about the Raman modes will be discussed later in the dissertation.) Recall that the only difference between the two sets of samples is the degree of order. The “ordered” samples are highly crystalline with DNA molecules aligned in crystalline order. Such crystalline samples have many intermolecular bonds between the aligned DNA molecules. In contrast, the disordered samples have the molecules in random orientation and are expected to have far fewer intermolecular bonds. The fact that
Figure 9 shows that only the crystalline DNA is in the A conformation provides strong support for the hypothesis that the A conformation is stabilized by intermolecular bonds. The amorphous samples with far fewer intermolecular bonds are not in the A conformation.

The next logical step is to probe the strength of intermolecular bonds. Lee et al.\textsuperscript{27} did this using Brillouin spectroscopy. Brillouin spectroscopy measures the speed of sound in a sample, providing an independent measure of the stiffness of the intermolecular bonds. Lee et al.\textsuperscript{27} took Brillouin spectra for phonons propagating both perpendicular and parallel to the helical axis.
One would expect the speed of sound to be greater for sound waves propagating along the helical axis since those sound waves are compressing the strong intramolecular bonds. Sound waves propagating perpendicular to the helical axis should move at a slower speed since the intermolecular bonds are expected to be weaker than the intramolecular ones. Surprisingly, the speed of sound in the perpendicular directions was found to be greater than in the parallel direction. This is a strong indication that the intermolecular bonds in DNA are very strong.

If the A conformation is stabilized by intermolecular bonds, then there should be a minimum strength of the intermolecular bond under which the A conformation is not observed. Can a critical value for the intermolecular bond to hold the DNA in the A

---

Figure 10 – Crystalline DNA is highly ordered. \( V_p \) represents speed of sound parallel to the helical axis and the other is the speed of sound perpendicular to the axis.
confirmation be observed? Lavalle et al.\textsuperscript{23} performed a lattice dynamical calculation for low frequency vibrations of DNA to address the question. The calculation of Lavalle et al.\textsuperscript{23} showed that all the force constants were independent of water content except for the intermolecular force constant, $k_{\text{IB}}$. The values of $k_{\text{IB}}$ for Na-, K-, Rb-, and CsDNA at various water contents are shown in Figure 11.

![Figure 11– Intermolecular force constant versus relative humidity for NaDNA (circles), KDNA (squares), RbDNA (diamonds) and CsDNA (triangles) Data from reference 23.](image)

Recall that the hypothesis is that the A conformation of DNA is unstable if the intermolecular bond $k_{\text{IB}}$ falls below some critical level. Also recall that DNA with the monovalent counterions, Na, K, Rb, and Cs undergo the A to B transition at 92\%, 90\%, 90\% and 80\% relative humidity, respectively. This implies that the intermolecular bond weakens below this critical value at those humidities for those counterions. There is a line drawn in at 160 µdyn/Å. All DNA above the line are in
the A form and all DNA below this line are in the B form. Therefore, 160 μdyn/Å is the critical value for the intermolecular bond to stabilize the A conformation of DNA.

In a review of x-ray crystallography experiments\textsuperscript{28-34} calcium is found to interact with the phosphate groups and has also been located within the major and minor grooves in B DNA.\textsuperscript{30-31, 33, 35-37} Solar-Lopez et al.\textsuperscript{28} were able to locate 3 calcium ions in a dodecamer (DNA fragment of 12 base pairs), one serving as an intermolecular bond with neighboring DNA and the other two ions were in the major groove. Chiu et al.\textsuperscript{30} found that some of the calcium ions interacted exclusively with the phosphate backbone atoms while others penetrated into the grooves. However, the calcium located in the grooves is dependent on the sequences of the base pairs.\textsuperscript{30} Since the DNA samples in this work are taken from salmon testes, the base-pair sequences are not known. Additionally, calcium located in the grooves have been shown to result in intramolecular bending.\textsuperscript{29-30} Since this work focuses on intermolecular bonds (how DNA interacts with other molecules), the calcium at the periphery (those calcium located near the phosphates) are the ones of interest.

Grzeskowiak et al.\textsuperscript{29} shows the result of their crystallographic experiment in localizing the bridging of calcium ions between to DNA complexes. Their work clearly shows calcium serving as an intermolecular bond between neighboring DNA molecules.
Work\textsuperscript{28-34} showing that calcium lies in the proximity (within 5 Å) of the phosphate group suggests that the calcium lies toward the exterior and serves as intermolecular links. Recall, the critical, intermolecular strength of 160 µdyn/Å must be obtained to pull DNA into the A conformation. Since we know that the counterion mitigates the intermolecular bond, in the case of calcium, the strength of the bond is apparently below the critical value; thus, all the DNA stays in the B conformation. This could explain why none of the CaDNA adopts the A conformation.

2.C. DNA Conformation Via Raman Spectroscopy

Raman spectroscopy is an excellent tool for probing vibrational modes of DNA. Many of these modes have been assigned to specific molecular interactions.

Erfurth, Kiser and Peticolas\textsuperscript{38} showed that Raman spectroscopy can be used to determine the conformation of DNA. They found that a Raman mode is present at 807 cm\textsuperscript{-1} in A DNA. In B DNA, the 807 cm\textsuperscript{-1} mode is replaced by a mode at 834 cm\textsuperscript{-1}. Figure 12 shows Raman spectra from DNA in the A conformation and the B conformation.
Figure 12 – Raman spectra of wet spun calf thymus CaDNA free standing film at 81% (top) and wet spun calf thymus NaDNA free standing film (bottom) at 75% relative humidity.
Chapter 3

III. Experimental Data

3.A. Wet-Spun Films

Wet-spun films used in this research were made by Professor Allan Rupprecht at the University of Stockholm. Double stranded DNA is stable in water, with the presence of an electrolyte and at neutral pH. Also, DNA is precipitated in the form of a fibrous deposit by alcohol. The DNA wet-spinning process takes advantage of this occurrence.\(^{25}\)

A solution of salmon testes NaDNA from Fluka (1.3 mg NaDNA per mL in 150 mM NaCl + 3 mM Na\(_3\)-citrate + 0.05 mM Na-EDTA) is continuously extruded through a spinneret at a rate of 60 mL/h into a spinning bath. The spinneret, made of glass, resembles a small showerhead. It contains 1500 cylindrical holes each 60 microns in diameter and two millimeters in length. The bath is 80% ethanol and contains 150mM of NaCl. The DNA precipitates at the top of the glass column as soon as it
comes into contact with the spinning bath. This is shown in Figure 13. A stationary V-shaped fiber guide at the bottom of the column converges the DNA fibers into a bundle that is wound onto a rotating Teflon-coated glass cylinder which is 64 mm in diameter and is rotating at a speed of 20 revolutions per minute. This Teflon-coated glass cylinder is rotating approximately ten times faster than the DNA is coming from the spinneret. This difference creates a stress which causes the helical axis of the DNA to align in the direction of the applied stress. While rotating, the spool moves slowly back and forth in an axial direction much like thread on a spool. A deposit of parallel fibers of oriented DNA is built up to the desired width and thickness.

An optical and electron microscopy study of these films shows that they are indeed composed of bundles of fibers about three microns in diameter. It also shows that each fiber bundle is composed of microcrystalites about 20 nm in diameter and macroscopic in length. The helical axes of the microcrystalites are highly oriented. The films are approximately 30 microns thick.
3.A.a. Bathing

The cylinder containing the deposit of parallel fibers of salmon testes NaDNA are bathed for two weeks at room temperature in three consecutive baths of 80% ethanol containing about 0.4 M CaCl$_2$ at first. This was done three different times with the
only difference being that the last bath contained either 0.03M, 0.12M or 0.24M of CaCl₂. This results in three different samples of highly ordered CaDNA films. The films are solid and free standing. They resemble small pieces of Scotch Tape. After the final bath, the samples were brought to +5 degrees Celsius as a preliminary to the drying process. Recall, Rupprecht noticed that the best results of x-ray crystallography experiments were taken on films that were bathed in the excess NaCl concentration of 0.03M. This corresponds to one excess ion pair per 10 base pairs. The reason for using three different molar bath concentrations of CaCl₂ is to see if additional counterions changes the stability of the DNA molecule in any manner.

Figure 14 - Bathing Process for Wet-Spun, Ca-DNA films.

3.A.b. Drying
The cylinder was placed on a holder while excess alcohol from the bathing solution was removed with absorbent paper. Next, the cylinder was placed in an empty dessicator still at +5 degrees Celsius. In one or two days some of the water and ethanol are released from the DNA deposit on the cylinder. The liquid that condensed on the dessicator walls is removed and a container of a saturated NaCl solution is added to the dessicator to further dehydrate the samples. Finally, the dessicator is brought to room temperature.\textsuperscript{25}

Drying Process

![Diagram of the drying process](image)

Figure 15 – Drying of Calf–Thymus, Wet-Spun, Ca-DNA Film

Next, the cylinders are removed and placed in a desiccator that contains a few grams of silica gel. The alcohol and some of the water are removed through absorption into the gel causing the DNA fibers to fuse together into thin films. The films are then
slowly dried and the DNA fibers coalesce into highly ordered films of CaDNA which can then be cut from the Teflon spool using a razor blade.\textsuperscript{25}

3.B. Swelling Experiment

Measurements are made on small pieces of highly oriented films to determine the changes in the intermolecular spacing as a function of relative humidity. To collect the swelling data, CaDNA wet-spun films are cut and mounted with a quick dry epoxy onto two steel prongs such that the samples are suspended inside an airtight polycarbonate box. The removable top and bottom of the box are made of transparent polycarbonate and the sides are made of brass. One of the steel rods is soldered and permanently attached to the brass side while the other is secured by a mechanical feed-through that permits small manipulations of the DNA film for the purpose of better observing the sample under a microscope. Two such boxes are used; one holds five samples and the other holds four. Samples are mounted until the boxes are full. The epoxy was given a day to dry then, 16 cuvettes containing Drierite were placed inside the polycarbonate box with the DNA samples. The top was then attached and sealed. See Figure 16.
Photos were taken of the samples in order to identify the positions on the films where measurements could be made. One of these pictures is shown in Figure 17.

Figure 16 – Chamber that contained the DNA samples.
These positions were marked on the photos to map the locations of measurements. The first measurement of the size of the films at zero percent relative humidity was taken a day after the Drierite was added. Subsequent measurements of the film sizes were made until the films reached equilibrium. After obtaining these reference points, the Drierite was removed and replaced with different salt solutions that pertained to various relative humidities. The relative humidity is controlled by having a saturated salt solution of the appropriate inorganic salt solution exposed to the same air in which the samples are kept. For example, the air above a saturated solution of sodium bromide is at 59% relative humidity at 23°C. Table 3 shows the saturated salt solutions used to control the relative humidity in our experiments.
Table 3 – Inorganic saturated solution and relative humidity in air above it.\textsuperscript{40}

<table>
<thead>
<tr>
<th>Relative Humidity</th>
<th>Salt Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Drierite</td>
</tr>
<tr>
<td>23</td>
<td>Potassium Acetate</td>
</tr>
<tr>
<td>33</td>
<td>Magnesium Chloride</td>
</tr>
<tr>
<td>54</td>
<td>Magnesium Nitrate</td>
</tr>
<tr>
<td>59</td>
<td>Sodium Bromide</td>
</tr>
<tr>
<td>75</td>
<td>Sodium Chlorate</td>
</tr>
<tr>
<td>80</td>
<td>Ammonium Sulfate</td>
</tr>
<tr>
<td>84</td>
<td>Potassium Bromide</td>
</tr>
<tr>
<td>88</td>
<td>Barium Chloride</td>
</tr>
<tr>
<td>92</td>
<td>Sodium Bromate</td>
</tr>
<tr>
<td>93</td>
<td>Ammonium Phosphate</td>
</tr>
<tr>
<td>95</td>
<td>Sodium Phosphate</td>
</tr>
</tbody>
</table>

The samples were numbered according to their placement in the chamber and in which chamber they were kept. Measurements of the widths of the films were made at the same reference points. This is the procedure used to determine the expansion of DNA due to water absorption. The results of the expansion of the films versus relative humidity for films bathed in excess salt concentrations of 0.03M CaCl\textsubscript{2} are averaged and shown below. Only the samples bathed in the 0.03M baths are shown. Micro-crystals were observed in the films bathed in salt concentrations of 0.12M and 0.24M of CaCl\textsubscript{2} and therefore one could not tell if the swelling was due to absorption into the films or into the crystals.
Figure 18 - Shows the average swelling of water into several (six) Ca-DNA films that was bathed in an excess salt solution of 0.03M of calcium chloride. This is compared with swelling data of monovalent films (NaDNA, KDNA, RbDNA and CsDNA) taken from Lavalle44.
3.3.1 Raman Experiment

Wet-spun, free-standing CaDNA films and NaDNA films were cut into pieces approximately 2 mm by 6 mm in dimension and mounted with a quick setting epoxy onto small wire circles. Salt solutions listed in Table 3 were used to control the relative humidity. The configuration of the samples is shown in Figure 19.

Figure 19 – Physical layout of the DNA samples for the Raman experiments.
The wire loops with the mounted CaDNA films and NaDNA film were then inserted into the glass vial and sealed. This ensures that all the samples were subjected to the same relative humidity. Samples were then stored for 1-2 weeks to establish equilibrium conditions within the samples.

Spectra were taken using a LabRAM which is an integrated Raman system model made by HORIBA Jobin Yvon. This system consists of a Pentium computer with Labspec software and VITEC video card for the digitalization of the TV image.

An adapter was added to change the angle of the objective by 90 degrees so the samples could remain upright.

Figure 20 – Close-up of Raman scattering geometry.
An objective of 50x magnification was then attached to this adapter. The laser was turned on and the power was measured through the objective at approximately 3-4 mW at the sample. Laser powers below 8 mW have been shown to be safe for Raman experiments.²² All experiments used a backscattering geometry.

Raman experiments were taken by using the TV monitor to focus the laser on the sample. Spectra were then acquired. The instrument was first calibrated by measuring the 520.9 cm⁻¹ Raman mode of crystalline silicon along with its Raleigh peak at 0 cm⁻¹. The DNA spectra are shown in Figures 21-23.
Figure 21 - Raman spectra of Ca-DNA bathed in excess CaCl$_2$ concentration of 0.03M at various humidities.
Ca-DNA (0.12 M bathing solution)

Figure 22 - Raman spectra of Ca-DNA bathed in excess CaCl₂ concentration of 0.12M at various humidities.
Figure 23 - Raman spectra of Ca-DNA bathed in excess CaCl$_2$ concentration of 0.24M at various humidities.
Chapter 4

IV. Analysis

4.A. Raman Experiment

4.A.a. Analysis of Raman Experiment

The 787 cm$^{-1}$ peak corresponds to a vibrational mode common to DNA in all conformations and the 835 cm$^{-1}$ peak is the B-conformation marker band. The intensity of each peak was found by integrating the area under the curve. These intensities along with the error is listed in Data Table 4. Because these peaks overlap, a difficulty exists in separating them. For this reason, to avoid error caused from the overlap in the peaks, only the low wavenumber half of the 787 cm$^{-1}$ frequency peak was integrated and the high wavenumber half of the 835 cm$^{-1}$ frequency peak was integrated.
The ratio of the 787 cm\(^{-1}\) mode to the 835 cm\(^{-1}\) mode gives an evaluation of the relative amount of B DNA in the wet spun films.

4.A.b. Error Analysis

The intensity of these peaks in our Raman spectra can be found through integration.\(^{41}\)

\[
A_p = \int_{-\infty}^{\infty} y_p(\text{cm}^{-1}) d(\text{cm}^{-1})
\]

In Raman spectroscopy the number of photons excited are what gives the intensity of the peaks. As this is a counting experiment it follows the same error rules as other methods subject to Poisson statistics.\(^{41}\) This also means that the uncertainties in intensities under the peaks is given by:

\[
\sigma_p^2 = A_p
\]

where \(A_p\) is the intensity of the peak and \(\sigma_p\) is the uncertainty in intensity. Because this work is interested in the intensity of the 835 cm\(^{-1}\) peak relative to the 787 cm\(^{-1}\) peak, we take the ratio of these two intensities. The relative uncertainty then is given by:

\[
\frac{\sigma_R^2}{R^2} = \frac{\sigma_{787}^2}{A_{787}^2} + \frac{\sigma_{835}^2}{A_{835}^2}
\]

where \(R\) refers to the ratio of peak intensities, \(\sigma_R\) refers to the uncertainty in the ratio, \(A_{835}\) is the intensity under half of the peak at 835 cm\(^{-1}\) and \(A_{787}\) is the intensity under half of the peak at 787 cm\(^{-1}\), \(\sigma_{787}\) is the uncertainty in the intensity of half of the
peak located at 787 cm$^{-1}$ and $\sigma_{835}$ refers to the uncertainty in the intensity under half of the peak at 835 cm$^{-1}$.

4.B Thermogravimetric Experiment

4.B.a. Data Collection of Thermogravimetric Experiment

In these experiments, samples of guanosine are rapidly brought to and then held at, a temperature slightly below the temperature at which the water of hydration is driven off of the DNA. The amount of water which is driven off at any moment is determined by carefully measuring the mass of the samples. The closer the experimental temperature is to the transition temperature, the more quickly the sample is dehydrated.

For these experiments, nucleosides (a base and a sugar) are studied. This is done since this phosphate group is known to be highly hydroscopic. By omitting the phosphate group, the details of hydration of the base and sugar can be studied. For the initial experiments, the nucleoside guanosine composed of guanine and a ribose sugar (called rG here) are studied.

Powder form of rG was kept in a dessicator at 59% relative humidity until equilibrium is achieved. These pristine samples are then heated up at a rate of 5°C per minute to 180°C and cooled. This initial heating destroys the crystallinity in the
samples and makes them disordered. This is believed to make the samples more biologically relevant. The samples are then placed back in the dessicator at 59% rh. By keeping the rG at such a low relative humidity only primary hydration can occur. Samples are held at 59% relative humidity for at least a week before an experiment is performed. For an experimental run, the samples are brought up to the experimental temperature (37°C or 42°C) rapidly (at a rate of 5°C per minute) and held at that temperature for 150 minutes to remove all the water. Figure 24 and 25 show the fraction of water being driven off rG.
Figure 24 – Shows the percent weight loss of rG at 37°C. The open circles show the fraction of water being driven off. The solid line is the curve fit to the data points.
Figure 25 – Shows the percent weight loss of rG at 42˚C. The open circles show the fraction of water being driven off. The solid line is the curve fit to the data points.
4.B.b. Analysis Thermogravimetric Experiment

As expected, at the higher temperature of 42°C the rate at which water is lost is higher than at the lower temperature of 37°C. The activation enthalpy of rG is calculated using Bagley’s method. In his method, curves describing general nucleation and growth formation were fit to these data using the equation:

$$X_c = 1 - \exp\left[-(kt)^n\right]$$

where $X_c$ is the fraction transformed; (in this case, this pertains to the amount of water being driven off); $k$ a kinetic constant; $t$ the time and the exponent $n$ is determined by the transformation mode. The values for k and n are listed in Table 4.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>K</th>
<th>$\sigma_k$</th>
<th>N</th>
<th>$\sigma_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>rG 42°C</td>
<td>.084</td>
<td>.0006</td>
<td>1.504</td>
<td>.0248</td>
</tr>
<tr>
<td>37°C</td>
<td>.055</td>
<td>.0004</td>
<td>1.344</td>
<td>.019</td>
</tr>
</tbody>
</table>

The activation enthalpy ($\Delta H$) is calculated from the temperature ($T$) dependence of $k$ and the Arrhenius relation,

$$k = k_o \exp\left[-\frac{\Delta H}{RT}\right],$$

where $k_o$ is a constant. From the $k$ values given in Table 5, it is determined that the activation enthalpy for rG is 16.487±0.402 kcal/mole or 0.715±0.017 eV/molecule.
Chapter 5

V. Results and Discussion

5.A. Swelling Experiment

Up to about 80% relative humidity, the swelling experiment shows small swelling which implies a small absorption of water for CaDNA films that were bathed in salt concentration of 0.03M CaCl₂. Such a small absorption is consistent with primary hydration. Beyond 80% relative humidity, a large swelling of the films is observed. This is consistent with a secondary hydration. CaDNA films swell similar to wet-spun DNA films that contain monovalent counterions. This suggests that intermolecular spacing increases as hydration occurs in CaDNA films similarly to those films which contain monovalent counterions.

It is known that water activity affects the conformation and stability of DNA. It is also known that the number of water molecules per base pair at the A to B transition varies according to the identity of the counterion. This is shown in Table 2. Simply
put, reduced water activities due to reduced relative humidities create an environment more favorable for A DNA. As water is introduced via increasing the relative humidity, B DNA becomes more stable. In the CaDNA films, the A conformation is never observed even at low relativity humidity.

Alden and Kim\textsuperscript{43} offer some understanding in the reduced number of water molecules associated with A DNA compared with B DNA. It is suggested that the more exposed sugar ring of A DNA increases a hydrophobic area. This hydrophobic area could explain why lower humidities are associated with the A conformation. Schneider et al.\textsuperscript{44} go on to point out a subtle, structural effect. The A conformation of DNA is more compressed than the B conformation; therefore, the interphosphate distances along the backbone are also compressed. This reduction between the phosphate groups enable favorable water bridges to be created between the oxygen atoms on adjacent, intrastrand phosphate groups. The water bridges in A DNA are believed to be an important contribution to the stability of A DNA while at the same time it explains the reduction in waters. In B DNA, the phosphate groups are hydrated individually. Egli et al.\textsuperscript{45} observed, using x-ray crystallography, that fewer water molecules are indeed necessary to hydrate the closely spaced phosphate groups in A-DNA as compared to the individual hydration shells phosphate groups in B DNA.

The mobilities of water molecules strongly associated with DNA are much reduced and occupy well defined hydration sites in the major and minor grooves and at the
phosphate oxygens. They are localized enough to be observed by x-ray and neutron diffraction. A “spine of hydration” of single water molecules in the minor groove of B DNA was observed by Kopka et al.\textsuperscript{46} in 1983 and was observed in A DNA by Kennard et al.\textsuperscript{47} in 1986.

Feig and Pettitt\textsuperscript{48} created a molecular simulation of DNA hydration around A and B DNA incorporating many experimental observations. These simulations were done for both A and B DNA two times.

In A DNA, two important observations are made. First, sodium ions associate strongly near guanine and adenine bases resulting in a rearrangement of the hydration pattern. Extensive water networks are formed to accommodate the ionic solvation shell. Second, the ions stabilize the hydration shell along the water bridges between the adjacent phosphates in the backbone by fixing the waters more rigidly in space.

In B DNA, the water structure was also observed to be disrupted in the presence of ions. This disruption, however, occurs less frequently than in A DNA and it occurs in the minor groove near the bases adenine and thymine. In the molecular dynamic simulations, the sodium ions cause the waters to reorient such that ribose sugars are exposed for ion interactions.\textsuperscript{51} These interactions correlate with an even more decreased groove width that further enhances the stability of B DNA! Experimental evidence that suggests some of the water in the spine of hydration is replaced by
sodium corroborates this claim. Also, in a simulation using Mg$^{2+}$ ions a general reduction in water mobility was also observed.

What is most interesting about Feig and Pettitt’s molecular simulation is the view that the presence of ions cause a rearrangement and immobilization of water molecules near DNA. They show that the first layer of water molecules are not replaced by additional ions but simply rearranged. The main effect of ions on water activity around DNA is reducing water mobility but not the number of waters.

5.B. Raman Experiment

Raman spectroscopy has been used to probe the amount of B conformation as a function of relative humidity in wet-spun films of CaDNA which contain varying amounts of CaCl$_2$. Our experiments show that CaDNA is in the B conformation from 98% relative humidity down to 75% relative humidity and is disordered at lower humidities.

The ratio of the relative intensities of the 787 cm$^{-1}$ mode to the 835 cm$^{-1}$ mode, indicates the relative amount of B DNA. Near 80% relative humidity a maximum of B DNA is observed in our samples. This is shown in Figure 26.
Because the samples are bathed in CaCl₂ of the varying quantities, 0.03M, 0.12M and 0.24M, it is assumed that the excess CaCl₂ within the samples increases. It can be seen in Figure 26 that at relative humidities 75%, 80% and 81% as the CaCl₂ increases so does the relative amount of DNA in the B conformation. Above 81% relative humidity, this is no longer true. Falk, Hartman and Lord¹² showed that water is
critical to the formation of the structure of DNA. For this reason, it is expected that as hydration increases, the relative amount of DNA in the B conformation should also increase. However, the decline in relative amount of B DNA above 81% relative humidity is not expected. Although the amount of excess CaCl₂ is changing, the percent relative humidity where we see the largest number of DNA in the B conformation remains 81%. From the work of Lavalle¹⁴, it is known that at 81% relative humidity, there are 22 water molecules per base pair.

It should be noted that the amount of CaDNA in the B conformation is about twice the amount as ever has been observed at 81% relative humidity. This enhancement of the B conformation suggests that something about the combination of CaCl₂ and water in the film stabilizes the B conformation.
In Figure 27 we compare our results with data published from other authors.\textsuperscript{22, 38, 54, 56-58} Note that the data at 105 percent relative humidity there are 5 data points. These data were taken from DNA in solution. In order to make a comparison, we plot it at 105\% relative humidity, even though relative humidity does not apply. We find our results correspond reasonably with those of our colleagues.
The counterion calcium is divalent unlike the monovalent counterions of sodium, potassium, rubidium, and cesium on which there is considerably more information. Due to calcium being divalent we expect the geometry of its position relative to the DNA to be different compared to the monovalent counterions since half as many counterions are necessary for neutrality.

Bartenev et al.\textsuperscript{51} used x-ray crystallography on CsDNA and determined that half of the Cs, a monovalent counterion, fits into the major groove of the DNA while the other half is deep inside the minor groove.

Recently, Das et al.\textsuperscript{52} were able to probe monovalent (Rb\textsuperscript{+} and Na\textsuperscript{+}) and divalent counterions (Sr\textsuperscript{2+} and Mg\textsuperscript{2+}) distribution around DNA using small-angle x-ray scattering. They found that indeed half as many Sr\textsuperscript{2+} and Mg\textsuperscript{2+} counterions are needed to neutralize DNA as Rb\textsuperscript{+} and Na\textsuperscript{+} counterions. Also, modulating ion scattering factors and interchanging ion identities they produced direct measurements of the scattering signal. The quality of their data permit quantitative tests of counterion distributions that were calculated from atomic scale models. They determined possible locations of the divalent counterions theoretically using the nonlinear Poisson-Boltzman (NLPB) model and calculating the distribution of divalent counterions. From their small-angle, x-ray scattering measurements they found, “unprecedented, quantitative agreement between the data and atomic-scale NLPB calculations”.\textsuperscript{52}
Clearly the physical geometry of divalent counterions is different than monovalent counterions in regard to where they bind to DNA. Only half as many divalent counterions are necessary for charge neutrality as monovalent counterions. Recall, Lavalle et al.\textsuperscript{23} found a critical value necessary for A DNA to exist. Since A DNA is not observed in CaDNA then it is concluded that the overall strength of the intermolecular bonding in CaDNA is weaker than for monovalent DNAs.

In addition, we have the molecular simulations of Feig and Pettit\textsuperscript{48} that suggest the arrangement of waters is altered in the presence of a counterion. Analysis of our Raman spectra show that a maximum in the number of DNA in the B conformation exists at 81\% relative humidity. Perhaps, in the presence of calcium there exists a favorable orientation of water at this relative humidity that stabilizes the B conformation. The more calcium that is available, the more B DNA would appear. Additional waters could interfere with such an orientation and cause the number of DNA in the B conformation to diminish.

5.C Thermogravimetric Experiment

Using the method proposed by Bagley\textsuperscript{42} we were able to measure the activation energy of rG. We found it to be $0.715\pm0.0174$ eV/molecule. This has been previously measured by means of differential scanning calorimetry. Cavanaugh and Lee\textsuperscript{53} have previously measured the activation energy of rG to be $0.683\pm0.090$ eV/molecule. Below are the results of our initial experiments.
Excellent agreement between the two techniques is noted.
Chapter 6

6. Summary

The results of the swelling experiment show that intermolecular spacing of DNA within CaDNA wet-spun films increases similarly to films with a monovalent counterion. The Raman experiment shows that CaDNA remains in the B conformation at and above 75% relative humidity. It never undergoes a transition to the A form even at low humidities. It is also observed that the addition of CaCl₂ to these films produces an enhancement in the amount of B DNA at about 81% relative humidity. Films bathed in 0.24M CaCl₂ showed as much as 75% of the CaDNA in the B conformation. This is twice as much as previously observed. From Lavalle’s¹¹ work, it is known that at 81% relative humidity there are approximately 22 water molecules per base pair. A maximum in the amount of B DNA at 81% relative humidity suggests that the combination of CaCl₂ and water enhance the stability of the B DNA.

The thermogravimetric experiment validates Bagley’s⁴² method of finding the activation enthalpy of water and guanosine. Activation enthalpy is 16.487±0.402
kcal/mole or 0.715±0.0174 eV/molecule. This significantly reduces the error bars from the previous measurement made by Cavanaugh and Lee$^{53}$. 
References


3. “Perhaps I should have asked Rosalind’s permission, and I didn’t. Things were very difficult. Some people have said I was entirely wrong to do this without her permission, without consulting her, at least, and perhaps I was…If there had been anything like a normal situation here, I’d have asked her permission, naturally, though if there had been anything like a normal situation the whole matter of permission wouldn’t have come up… I had this photograph, and there was a helix right on the picture, you couldn’t miss it. I showed it to Jim, and I said, ‘Look, there’s the helix, and that damned woman just won’t see it.’ He picked it up of course.”

(Interview with Maurice Wilkins, June 15, 1970, “Rosalind Franklin and DNA” by Anne Sayre, Norton 151 1975)


