

Kinetics of DNA Duplexing in Various Ionic Strengths Using Stopped-Flow Spectroscopy

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Submitted to the Allegheny College Department of Chemistry in partial fulfillment of the requirements for the degree of Bachelor of Science

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I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code and to maintain the integrity of both myself and the College as a whole.

Pledge: _____

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Abstract

Stopped-flow spectroscopy was used to obtain rate constants for DNA duplexation in various NaCl buffer concentrations. DNA duplexation was observed for complementary DNA strands consisting of eight bases (DNA1: 5'-GTGCTGTG-3', DNA2: 5'-CACAGCAC-3') with equal concentrations of both strands in second-order reaction conditions. DNA concentrations ranged from 1 to 7 μM in NaCl buffer concentrations of 0.048 to 1.0 M. Data was fitted to a second-order integrated equation. It was found that the observed rate constants for DNA duplexation increase with increasing NaCl buffer concentrations and also with increasing DNA concentrations. Results showed a positive, linear relationship between NaCl concentrations and the rate constants for DNA duplexation, given by $k = 11.89 [\text{NaCl}] + 0.9018$. The results are ambiguous with respect to support for the Debye-Huckel theory. Another possible theory is the tight binding model, in which ions directly coordinate to the DNA strands. Furthermore, it is hypothesized that a decrease in the activation barrier due to ionic strength could explain the positive, linear relationship between salt concentrations and rate constants for DNA duplexation.

Introduction

Duplex formation by complementary DNA strands is a fundamental biochemical process. More specifically, the salt dependence of duplex formation by short nucleic acids is important for the prediction of nucleic acid structure and dynamics under different buffer conditions.¹ An understanding of salt effects is also important for the prediction of the influence of salt on processes that involve formation of short duplex regions. RNA processing and control of gene expression by “antisense” nucleic acids are just two examples. For instance, RNase P, which is a ribozyme that cleaves off the precursor sequence of RNA on tRNA, and the catalytic activity of the self-splicing RNA from *Tetrahymena thermophila* has been shown to depend on salt.¹ In addition, effects of site-directed mutagenesis that disrupt base pairing can also depend on salt. While studying the self-splicing RNA from *Tetrahymena thermophila*, it was found that activity ceased at 2 mM Mg^{+2} because the magnesium cation disrupted conserved pairs in the self-splicing RNA.¹ However, this was not observed at 10 mM Mg^{+2} in the presence of 0.1 M $(\text{NH}_4)_2\text{SO}_4$.

Thus, although many biochemical processes are influenced by ionic interactions, little research has been done regarding the kinetics of DNA duplexation under various salt concentrations. Therefore, the goal of this study is to determine the rate constant for DNA duplexation of two complementary DNA strands consisting of eight bases in various salt concentrations. All extraneous factors, such as temperature, will be held constant. A stopped-flow instrument will be used with equal concentrations of both strands under second-order reaction conditions.

DNA strands

In this study, the chosen complementary DNA strands are the T strand: 5'-GTGCTGTG-3' and the A strand: 5'-CACAGCAC-3'. These strands were chosen because of their great thermodynamic stability, and hence large melting temperatures. The two complementary strands are beneficial for determining the rate constant because they have a greater thermodynamic probability of forming duplexes than other less thermodynamically stable strands. These strands were also chosen because it is highly unlikely that base pairs will form within each strand. For example, the T strand has GTG bases at each end, which prevents formation of Watson-Crick base pairing and hence deters formation of a hairpin, which would prevent DNA duplexation between the two complementary DNA strands. Also, the fact that the DNA strands only consist of eight bases reduces the possibility of incorrect base pairing. For instance, for the T strand, and likewise the A strand, there are three possible GT pairings, two of which are wrong. The wrong pairings only consist of two base pairs though, which still allows for the strands to find the correct base pairings to form the full duplexed DNA. Furthermore, these wrong pairings would result in mismatches after two incorrect base pairings. The potential mismatches are C-A, G-T, C-C, and G-G. It was found that the canonical G-C base pair is 2.5-2.9 kcal/mol more stable than its mismatched counterpart G-G base pair and 3.6-8.0 kcal/mol more stable than the C-C base pair.² Because the mismatches are preceded by only two correct base pairs, these mismatches cause the DNA strands to break apart and form the full duplexed DNA that consists of eight correct base pairs. Furthermore, G-T and C-A mismatches were found to exist only if there were significant deformations in the DNA structure. For example, a G-T mismatch of MutS could only exist when a 60°

bend into the major groove was allowed.³ Because the DNA strands being studied are only eight base pairs, significant conformation changes are unlikely because this would result in an overall higher ΔG value, and thus destabilization of the DNA duplex.

Kinetics

The Wetmur-Davidson mechanism of duplexation of complementary DNA strands is depicted in Scheme 1.⁴

Scheme 1



First, short stretches of polymer strands align to form a nonbonded complex.⁴ This is followed by initial base pair formation and then formation of adjacent base pairs until a stable nucleus is present. Formation of the nonbonded complex is a pre-equilibrium step, but after the nucleus is formed, which was found to consist of either two or three base pairs,⁵ forward rates for additional adjacent base pairs are faster than reverse rates for breakage of base pairs. Thus, the helix “zips up” from the nucleus. This mechanism is further illustrated in Figure 1.

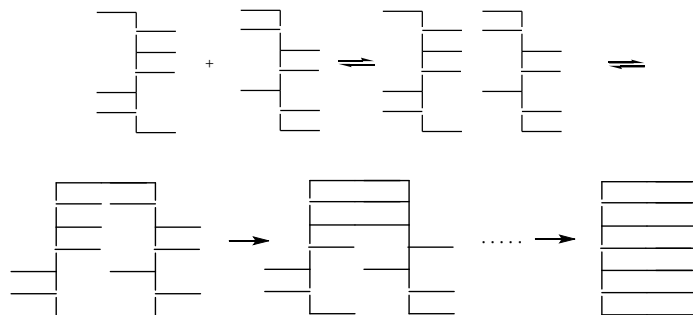


Figure 1
Mechanism of formation of DNA duplex

In this mechanism there are two rate constants, one for formation of the nucleus and one for “zippering up” of the helix. It has been proposed that the formation of the nucleus is not sensitive to ionic strength because the charge density for both the nonbonded complex and the nucleus is not significantly different.⁶ However, “zippering up” of the nucleus is dependent on ionic strength because it requires that phosphate electrostatic repulsions be overcome to form the duplexed DNA.

Another mechanism has been proposed that does not involve any intermediates, but instead assumes a two-state transition, as depicted in Scheme 2.

Scheme 2



This mechanism begins with two or three base pairs pairing and unpairing in rapid equilibrium.⁷ When an additional critical base pair is added, the helix subsequently forms base pairs until the DNA is fully duplexed. Thus, this mechanism follows a two-state transition because essentially there are only the reactants, in this case the two single-stranded strands, and the duplexed DNA product.⁴ This mechanism is simplified compared to the Wetmur-Davidson mechanism because there is only one rate constant corresponding to formation of the DNA duplex, which is when the critical base pair is added. NMR results verified that the transition from single strands to duplex is a two-state transition as depicted in Scheme 2.¹ This observation cannot rule out the Wetmur-Davidson mechanism though because if the pre-equilibrium step and steps after nucleation in the Wetmur-Davidson mechanism are very fast on the NMR timescale, then

the mechanism for DNA duplexation may just appear to look like a two-state, single step mechanism.

Effects of Salt

It has been observed that the rate of DNA duplexation increases with greater concentrations of salt, potentially to the third or fourth power.⁶ This has been explained by different theories. The Debye-Huckel theory rests on charge screening. Charge screening is the process in which sodium cations “screen” the phosphates’ negative charge and make it possible for short-range interactions such as hydrogen bonding and base stacking to stabilize the duplexed DNA.⁸ Sequences of nucleic acids are commonly treated as line charges that localize a sheath of cations nearby.⁹ Because the charge density is greater for duplexed DNA than single stranded DNA, cations are taken up in the formation of duplexed DNA. Therefore, increased concentrations of salt are expected to increase the rate of DNA duplexation because the salt stabilizes the molecule to a greater extent. When ionic strength effects are due solely to Debye-Huckel screening, the predicted dependence of rate on ionic strength, I , is given by equation 1:¹

$$\log k = \log k_0 + 1.02 Z_a Z_b \sqrt{I} \quad [1]$$

This equation predicts that the rate constant for DNA duplexation depends only on ionic strength and the charge on the two DNA strands.

The counterion condensation model is another theory used to explain the increased rate of DNA duplexation in salt. The binding of salt to the phosphate backbone

relieves electrostatic stress on the duplexed DNA that is due to the repulsive interactions of the phosphate groups, with fewer cations binding to the ends of the DNA strand than the interior.¹⁰ Thus, nucleic acids are highly charged systems and counterions condense on the negatively charged phosphates, imparting electrostatic stability to the system.

The extent to which counterions are localized near discrete groups, which is referred to as their “tightness” of binding, varies with both the nature of the charged groups and the counterion species.¹¹ At the one extreme, the counterion is directly coordinated to several neighboring charged groups. At the other extreme, the counterion is “delocalized” and not coordinated directly to any charged groups. In the case of DNA, each phosphate is rigidly held at a distance of only 7 Å from its nearest neighbor phosphate groups.¹¹ Because of the small distance between phosphates, the phosphate groups directly coordinate to cations, further supporting the prediction that increasing concentrations of salt should cause the rate constant to increase.

Other processes that relieve the phosphate electrostatic stress upon duplexation include transitions to conformations of lower charge density, such as DNA spontaneously straightening from a bent configuration and the helix to coil transition, more commonly known as DNA melting.¹¹ Because the DNA strands that are being studied are only eight bases long, DNA straightening will have little to no effect on the rate of DNA duplexation in different salt concentrations because the greater distance between the phosphates when straightened compared to bent is not significant. In addition, because DNA duplexation is being studied on a timescale of only a second, DNA melting can be

ignored because given the greater thermodynamic stability of duplexed DNA compared to single-stranded DNA, it will require a disturbance in the system for DNA melting to occur. Furthermore, CD and NMR spectra indicate that different salt concentrations have little effect on structures of duplex and single-stranded DNA consisting of ten bases or less.¹ Thus, the results are not complicated by additional salt-induced conformational changes. This supports that an increased rate for DNA duplexation is due to greater concentrations of salt and not due to some extraneous factor. This further validates that this is a suitable system for studying salt effects on DNA duplex formation.

The bases that make up the DNA strands can have an effect on how many cations bind to DNA and thus the extent of the effect of ionic interactions on the rate of DNA duplexation. In DNA, the fundamental building block is the nucleotide. A nucleotide of DNA consists of three elements: a nitrogen-containing purine or pyrimidine base, a phosphate group, and a deoxyribose sugar. Guanine and adenine are purine bases; whereas, cytosine and thymine are pyrimidine bases. Base pairing in DNA is very specific; adenine forms two hydrogen bonds with thymine and guanine forms three hydrogen bonds with cytosine. The backbone-to-backbone distance of a G-C pair is 1.08 nm and that of an A-T pair is 1.11 nm, as shown in Figure 2a and 2b respectively.

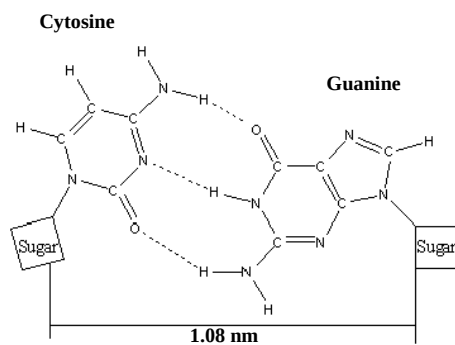


Figure 2a
Base pairing of Cytosine and Guanine,
with backbone-to-backbone distance indicated.

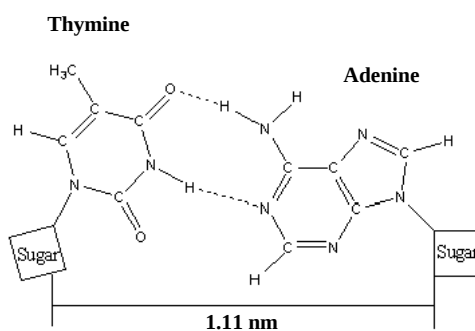


Figure 2b
Base pairing of Thymine and Adenine,
with backbone-to-backbone distance indicated.

Although the difference between backbone-to-backbone distances for the two pairs is minute, it was found that this small distance could affect the extent to which sodium binds to the phosphate backbone. It has been determined that the binding of sodium ions to DNA increases for sequences of higher G-C content.¹² This is speculated to be due to the smaller backbone-to-backbone distance for the G-C pair than compared to the A-T

pair, and hence a more collapsed structure and increased negative charge density for G-C rich sequences.

Based on the theories discussed above, it is expected that with increasing concentrations of salt, the rate of duplex formation will increase due to a decrease in repulsion between the negatively charged backbones. Furthermore, by utilizing equation [1], the log of the experimental rate constants can be graphed versus the square-root of the ionic strength to determine if the data can be explained by the Debye-Huckel model. If the data does not fit the Debye-Huckel model then the counterion condensation model may be used to explain why the rate of DNA duplexation increases with salt. In addition, the data should allow for the confirmation or refutation of the nucleation step and if it depends on ionic strength. If the graph of absorbance versus time fits an integrated second-order rate equation, then a two-state transition of the form depicted in Scheme 2 can be assumed. In this case, the nucleation step is either not occurring or occurring on a time-scale that is much faster than the subsequent steps in the mechanism. On the other hand, if the graph of absorbance versus time is bi-exponential, then it can be hypothesized that the mechanism for DNA duplexation involves formation of a nucleus as an intermediate. In this case, a rate constant for nucleation and a rate constant for zippering can then be determined. Whether the rates for nucleation and/or zippering depend on ionic strength can be suggested by looking at which rate constants change when the salt concentration is changed.

Experimental

Preparation of Melt Buffer

1 M melt buffer (1 M NaCl, 10 mM cacodylic acid, 1 mM ethylenediaminetetraacetic acid) was made and adjusted to pH 7 using a pH meter. Melt buffer without NaCl was also prepared and adjusted to pH 7 using a pH meter. By mixing the two melt buffers in different proportions, twelve melt buffers with sodium chloride concentrations in the range 0.048 to 1.0 M were made.

Preparation of DNA

DNA was purchased through Integrated DNA Technologies. DNA1 (the “T strand”) has the sequence: 5’-GTGCTGTG-‘3, and complementary DNA2 (the “A strand”) has the sequence: 5’-CAGAGCAC-‘3. 1 μ L of DNA was diluted 100X in deionized water and using ultraviolet/visible spectrometry at 80°C and 260 nm and also 280 nm, the absorbance of the DNA was measured. Using the absorbance and Hill coefficients, the concentration of the DNA was determined, as shown in Table 1.

	Absorbance at 260 nm	Absorbance at 280 nm	Hill Coefficient (L/mol*cm)	Concentration (μM)
DNA1	0.186	0.097	76100	186
DNA2	0.321	0.187	73300	347
DNA2-2	40.3	20.8	73300	417

Table 1. Table showing the absorbance at 260 nm and 280 nm, Hill coefficients, and concentrations for all three DNA strands used in the experiment. The absorbance was measured at 80°C, and Integrated DNA Technologies provided the Hill coefficients.

The DNA was speed vacuumed and then 1 mL of melt buffer was added at the salt concentration being tested. To determine the amount of DNA to be speed vacuumed, equation [5] was used, with C_1 equal to the desired concentration of DNA, V_1 equal to 1 mL of melt buffer, and C_2 equal to the concentration of the DNA in the stock solutions as shown in Table 1.

$$C_1V_1 = C_2V_2 \quad [5]$$

DNA strands were diluted to concentrations of 1, 2, 3, 5, or 7 μM . Solutions containing the DNA strands were stored at 5°C until the time of experimentation.

Experimental Setup

A stopped-flow instrument was used to determine the rate of DNA duplexation in various salt concentrations. The stopped-flow technique rapidly fires two DNA strands into a mixing device using driving syringes.¹³ The reactants are carried to an observation cell where they are illuminated by a deuterium light source and the absorbance is measured as a function of time. This technique allows for the study of DNA duplexation that occurs on the millisecond to second timescale. The setup of the stopped-flow spectroscopy is illustrated in Figure 3.¹⁴

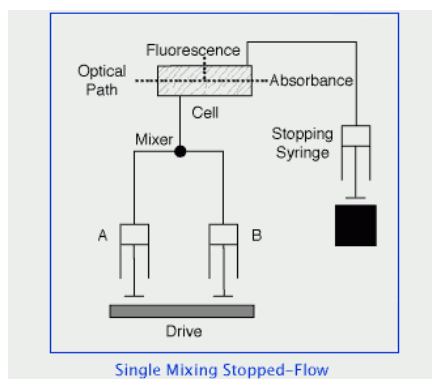


Figure 3
Stopped-flow Setup

Stopped-flow experimentation was conducted using KinTek Corporation Stopped-Flow Model SF-E100, shown in Figure 4.

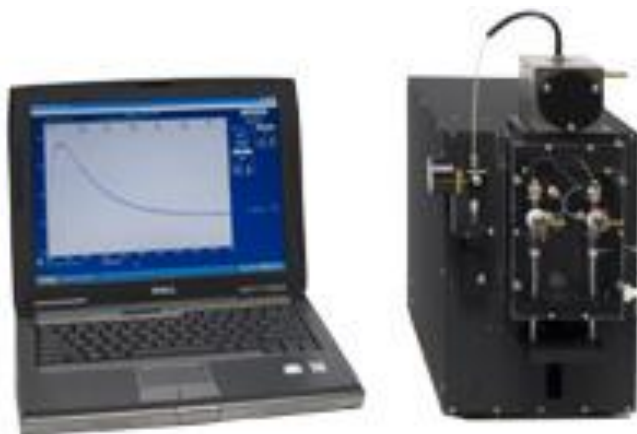


Figure 4
KinTek Corporation Stopped-Flow Model SF-E100

In this experiment, stopped-flow spectroscopy was performed at 5°C and monitored at wavelength 280 nm. The timescale was set to 1.0 second. For all melt buffer concentrations being tested, DNA1 reacted with DNA2, both at concentrations of 1, 2, 3, 5, or 7 μM . Approximately twenty shots were performed for each DNA concentration and then averaged together.

Results and Discussion

To determine the rate constant the rate law, $v = k[A][T]$, will be used.¹⁵ Because the concentration of the A strand is equal to the concentration of the T strand, the rate law can be simplified to [2], where $[SS] = [A] = [T]$:

$$v = k[SS]^2 \quad [2]$$

From the stopped-flow instrument, a graph of the absorbance of the DNA strand as a function of time is obtained. This data is fitted to the second-order integrated equation [3]:

$$abs = \frac{abs_o - abs_\infty}{1 + k[SS]_o t} + abs_\infty \quad [3]$$

An observed rate constant, equal to $k[SS]_o$, can be found from this fit, as shown in Figures 5 and 6.

As illustrated in Figure 5, the rate of decrease in absorbance for DNA duplexation increases with increasing concentrations of DNA. Because absorbance is directly related to the concentration of the single-stranded DNA, Figure 5 also shows that DNA duplexation is faster the greater the concentration of the DNA strands. Hence, 7 μM DNA duplexes faster than 3 μM DNA, which duplexes faster than 2 μM DNA. In addition, the solid lines for Figure 5 show the best-fit to equation [3]. These data fit a

second-order rate equation very well indicating that scheme 2 is a plausible mechanism for the duplex reaction.

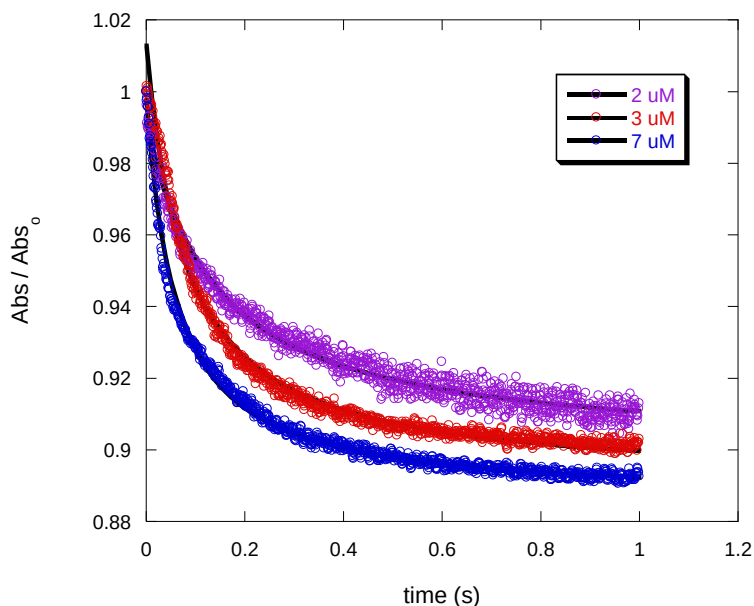


Figure 5. A graph displaying how the rate of decrease in absorbance for DNA duplexation increases with increasing DNA concentrations. Because absorbance is directly related to the concentration of the single-stranded DNA, the graph also shows that DNA duplexation is faster the larger the DNA concentration. DNA duplexation was tested in 0.2 M NaCl buffer. Purple dots represent DNA duplexation for 2 μ M DNA, red dots represent DNA duplexation for 3 μ M DNA, and blue dots represent DNA duplexation for 7 μ M DNA. The data for each DNA concentration was fitted to a trend line shown as solid lines over the data points using a second-order integrated equation.

Furthermore, DNA duplexation increases with increasing NaCl buffer concentrations as shown in Figure 6. The rate of decrease in absorbance is greater the larger the concentration of NaCl in the buffer. Since absorbance is directly related to the concentration of the single-stranded DNA, Figure 6 also shows that DNA duplexation is faster the greater the buffer concentration. Thus, DNA duplexation proceeds faster in 0.30 M buffer than in 0.25 M buffer, which progresses faster than in 0.20 M buffer. The solid lines in Figure 6 represent best-fits to equation [3] indicating that scheme 2 is a plausible mechanism at all salt concentrations.

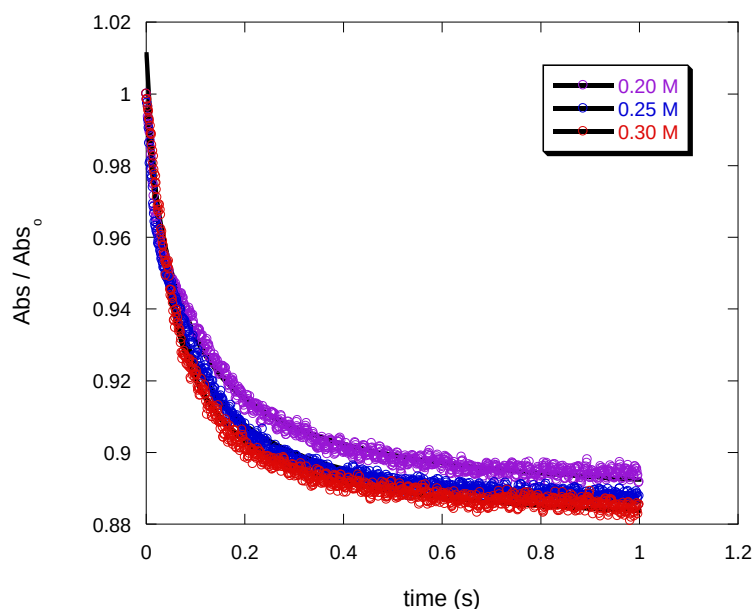


Figure 6. A graph displaying how the rate of decrease in absorbance for DNA duplexation increases with increasing NaCl buffer concentrations. Because absorbance is directly related to the concentration of the single-stranded DNA, the graph also shows that DNA duplexation is faster the larger the buffer concentration. DNA duplexation was tested for 5 μ M DNA. Purple dots represent DNA duplexation in 0.2 M NaCl buffer, blue dots represent DNA duplexation in 0.25 M NaCl buffer, and red dots represent DNA duplexation in 0.3 M NaCl buffer. The data for each NaCl buffer concentration was fitted to a trend line using a second-order integrated equation.

To obtain the true rate constant, the observed rate constant obtained at different DNA strand concentrations is plotted versus the corresponding concentrations of the DNA strands, as shown in Figure 7. The true rate constant k is related to the observed rate constant as shown in equation [4] and is therefore the slope of the best-fit line shown in Figure 7 for each salt concentration.

$$k_{\text{obs}} = k[\text{SS}]_0 \quad [4]$$

This was done for each buffer concentration being tested. As can be seen from Figure 7, there is a positive, linear relationship between k_{obs} and DNA concentration as

predicted by equation [4]. Thus, for each NaCl concentration, k_{obs} increases with increasing concentrations of DNA. Also, the slope of the best-fit line increases with increasing buffer concentrations. This indicates that the rate constant is a function of salt concentration.

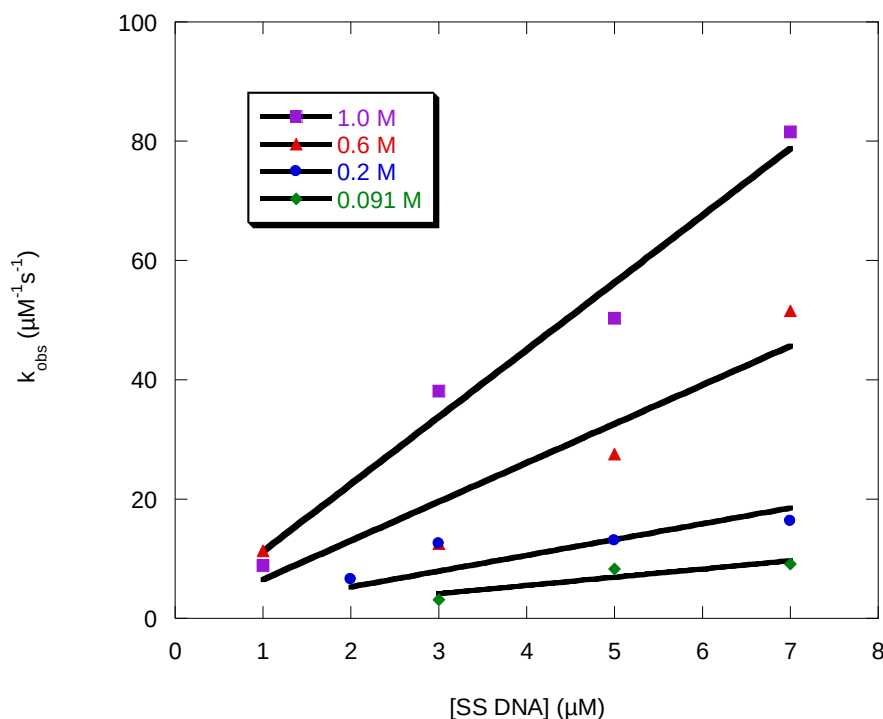


Figure 7. A graph displaying the positive, linear relationship between k_{obs} and DNA concentrations. Thus, for each buffer concentration, k_{obs} increases with increasing concentrations of DNA. Also, k_{obs} increases with increasing buffer concentrations at all concentrations of DNA being tested. Purple squares represent DNA duplexation in 1.0 M buffer, red triangles in 0.6 M buffer, blue circles in 0.2 M buffer, and green diamonds in 0.091 M buffer. For 1.0 and 0.6 M buffer, DNA duplexation was tested at DNA concentrations of 1, 3, 5, and 7 μM. For 0.2 M buffer, DNA duplexation was tested at DNA concentrations of 2, 3, 5, and 7 μM. For 0.091 M buffer, DNA duplexation was tested at DNA concentrations of 1, 3, 5, and 7 μM, but duplexation was not observed for 1 μM DNA. k_{obs} values for each buffer concentration were fitted to a linear trend line.

Rate constants were determined from the slopes of the best-fit lines in Figure 7.

This was observed in twelve salt buffer concentrations to determine the relationship

between NaCl concentration and the rate constant for DNA duplexation. As illustrated in Figure 8, there is a positive, linear relationship between salt buffer concentration and rate of DNA duplexation.

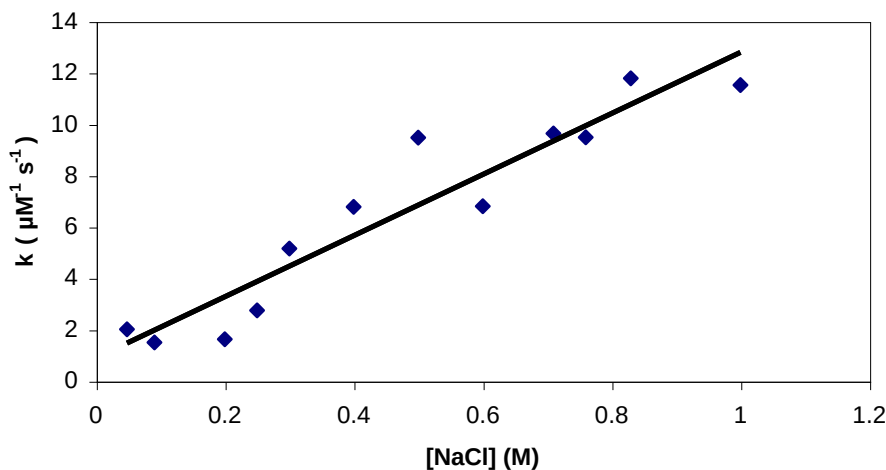


Figure 8. A graph displaying the positive, linear relationship between NaCl concentration and the rate constant for DNA duplexation. Rate constants were determined from the slopes of graphs of k_{obs} values versus the corresponding DNA concentrations. The best fit line shown, as a solid line, has the form $k = (11.89[\text{NaCl}] + 0.9018) \mu\text{M}^{-1} \text{s}^{-1}$

To determine if the data could be explained by the Debye-Huckel theory, using equation [1] and the rate constants obtained, $\log k$ versus the square root of the corresponding ionic strength of the salt buffer was graphed, as illustrated in Figure 9. Because Figure 9 ($R^2 = 0.859$) is not really any less linear than Figure 8 ($R^2 = 0.8936$), it might be proposed that the data follows the Debye-Huckel theory. Inserting the slope of Figure 9 into equation [1] and solving for the charge on the DNA strands gives an effective charge of -1.12 . A charge of -1.12 seems too low for DNA strands consisting of eight bases. However, the Debye-Huckel theory is for ideal solutions. Because the

system is highly charged, the solutions are likely to deviate from ideality. Thus, the results are ambiguous with respect to support for the Debye-Huckel theory.

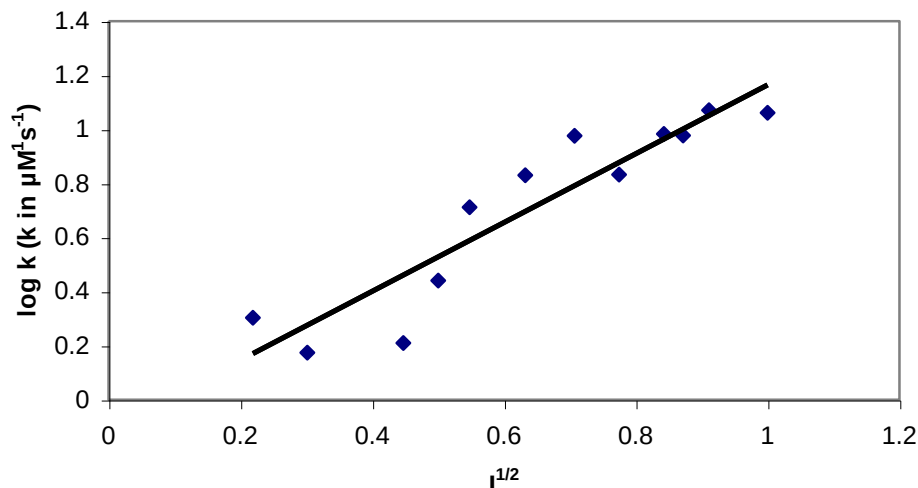
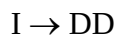


Figure 9. A graph displaying the relationship between the square root of the ionic strength of the buffer and the log of the rate constant for DNA duplexation. Rate constants were determined from the slopes of graphs of k_{obs} values versus the corresponding DNA concentrations. This was observed in numerous buffer concentrations to determine the relationship between the square root of the ionic strength of the buffer and log of the rate constant for DNA duplexation. The data was fitted to a linear trend line, shown as a solid line. The linear fit is represented by $\log k = 1.2721 I^{1/2} - 0.1078$.

Since these data fit equation [3] as shown in Figures 5 and 6 then the duplexation of the DNA strands is most likely to occur as a two-state transition as depicted in Scheme 2. On the other hand, the duplexation of the DNA strands could be more complicated and proceed through an intermediate, as depicted in Scheme 3 if the second step is much faster than the first step. In this case the first step is rate determining, and the observed rate constants correspond to the rate constant for this first step.

Scheme 3



To be consistent with the Wetmur-Davidson mechanism, the intermediate is associated with the nucleated strands.⁴ In this case, the first step would not be expected to depend on the salt concentration; whereas, the second step would depend on the ionic strength. The data shown in Figures 7 and 8 suggest that the Wetmur-Davidson mechanism can be ruled out because the measured rate constants are salt dependent.

Williams et al. reported the rate constants for DNA duplexation for two complementary strands with the sequence GCATGC in different salt concentrations but at higher temperatures than those performed in this experiment. Using equation [6],

$$k = Ae^{-E_a/RT} \quad [6]$$

Williams et al. results were recalculated to determine the corresponding rate constants at 5°C as shown in Table 2.

[NaCl]	k (s⁻¹ μM⁻¹) 5'-GCATGC-3' 5'-CGTACG-5' Williams et al.	[NaCl]	k (s⁻¹ μM⁻¹) 5'-GTGCTGTG-3' 5'-CACAGCAC-3' Experimental
1.0 M	4.047	1.0 M	11.511
0.15 M	2.686	0.2 M	1.6219
0.042 M	0.5518	0.091 M	1.4935

Table 2. Table comparing the rate constants for DNA duplexation for Williams et al. and those obtained in this experiment. All rate constants are those at 5°C. The DNA strands for Williams et al. consisted of 6 bases, whereas the DNA strands tested in this experiment consisted of 8 bases.

As can be seen from the table, our results agree with Williams et al. in that the rate constants for DNA duplexation increases with increasing salt buffer concentrations. As shown in Figure 8, our results produced a positive, linear relationship between NaCl concentration and the rate constant for DNA duplexation. On the other hand, the results of Williams et al. appear to display a tapering off effect at higher salt concentrations. Thus, these results may follow the Debye-Huckel theory. However, Williams et al. reported an activation energy of -6 kcal/mol. A negative activation energy is characteristic of a multiple step process. Our data support a one elementary step mechanism, which should have a positive activation energy. In our experiment there is a positive activation barrier,¹⁶ which may give more credibility to our results than those obtained from Williams et al. Also, results between the two may vary because Williams et al. studied DNA duplexation for six bases, whereas we looked at DNA duplexation for eight bases.

Another potential theory is the counterion condensation or tight binding model, in which ions directly coordinate to the DNA strands. Thus, the ions would decrease the charge on the DNA strands with the positive sodium ions essentially cancelling out some of the negative charge on the DNA backbones. A possible theory for the linear relationship between NaCl buffer concentrations and the rate constants for DNA duplexation could be that a less highly charged system may lower the activation barrier by stabilizing the transition state more than the single-strands are stabilized. This can be seen by adding a term for ionic strength, m , and the y -intercept, b , into equation [6] as shown below in equation [7]:

$$k = Ae^{-(E_o - RT \ln(am+b))/RT} \quad [7]$$

Then rearranging equation [7]:

$$k = Ae^{-E_o/RT} e^{\ln(am+b)}$$

$$k = k_o am + k_o b$$

where $k_o = Ae^{-E_o/RT}$, E_o = activation barrier at $[\text{NaCl}] = 0$, and a has units of concentration⁻¹. It is postulated that the activation barrier for DNA duplexation decreases with the log of ionic strength. Thus, manipulating equation [6] to account for a decrease in the activation barrier due to ionic strength could explain the direct, positive relationship that was observed for NaCl buffer concentrations and the rate constants for DNA duplexation. To verify this hypothesis, future research could be done to see if the activation barriers for duplexation decrease logarithmically with NaCl concentrations.

Appendix: Raw Data

0.048 M Buffer		k = 2.0106
[SS DNA] (μM)	k_{obs}	
1	-----	
3	2.1096	
5	6.0814	
7	10.152	
0.091 M Buffer		k = 1.4935
[SS DNA] (μM)	k_{obs}	
1	-----	
3	3.1426	
5	8.3096	
7	9.1167	
0.2 M Buffer		k = 1.6219
[SS DNA] (μM)	k_{obs}	
2	6.6134	
3	12.604	
5	13.033	
7	16.285	
0.25 M Buffer		k = 2.7549
[SS DNA] (μM)	k_{obs}	
1	4.8559	
3	20.414	
5	15.625	
7	24.818	
0.3 M Buffer		k = 5.148
[SS DNA] (μM)	k_{obs}	
2	9.9181	
3	22.539	
5	31.313	
7	37.432	

0.4 M Buffer		k = 6.7741
[SS DNA] (μM)	k_{obs}	
2	12.539	
3	30.771	
5	35.153	
7	-----	
0.5 M Buffer		k = 9.4695
[SS DNA] (μM)	k_{obs}	
1	5.9537	
3	24.613	
5	-----	
7	62.715	
0.6 M Buffer		k = 6.7944
[SS DNA] (μM)	k_{obs}	
1	11.326	
3	12.531	
5	27.557	
7	51.613	
0.71 M Buffer		k = 9.6277
[SS DNA] (μM)	k_{obs}	
2	8.8483	
3	49.279	
5	43.892	
7	-----	
0.76 M Buffer		k = 9.4846
[SS DNA] (μM)	k_{obs}	
2	10.569	
3	20.432	
5	51.768	
7	54.688	
0.83 M Buffer		k = 11.78
[SS DNA] (μM)	k_{obs}	
1	6.5079	
3	25.309	
5	53.627	
7	-----	

1.0 M Buffer		k = 11.511
[SS DNA] (μM)	k_{obs}	
1	8.91	
3	38.1	
5	50.33	
7	81.57	

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