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The Effect of Common Cations on DNA Degradation

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Summary

The process of DNA degradation is important to many scientific studies. Heat treatment is the standard procedure used to degrade genetic material by heating DNA-containing media past the DNA melting temperature, but certain chemical alternatives have been explored. More specifically, the presence of cations, such as Mg^{2+}, has been linked to increased stability of DNA molecules subjected to high temperatures. However, the possible effects of other ions have not been extensively studied; perhaps cations similar to magnesium may offer more versatile effects. This study examines the effects that NH_4^+, Ni^{2+}, and Li^+ have on the heat degradation and melting temperature of DNA. Magnesium chloride, magnesium sulfate, ammonium sulfate, nickel chloride, and lithium chloride solutions of different concentrations were mixed with DNA samples; the resulting mixture was heated and subsequently analyzed using gel electrophoresis. Treatment with NH_4^+ did not yield effects that significantly differed from Mg^{2+}, while Li^+ proved effective at preserving DNA even at high temperatures. Treatment with Ni^{2+} resulted in marked degradation of DNA. These results show that magnesium, ammonium, and especially lithium ions can be used for the preservation of DNA. The specific effects of Li^+ and Ni^{2+} on DNA are promising subjects for future research.

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Introduction

The denaturation and degradation of DNA are of high importance in scientific inquiry. Denaturation is the separation of double-stranded DNA (dsDNA) into two single strands (ssDNA) by the breaking of hydrogen bonds between individual base pairs. It is used in many different research techniques, notably in the amplification of DNA molecules through the polymerase chain reaction, or PCR, and is achieved by heating the DNA material higher than the melting temperature (T_m) of the molecule. T_m is the temperature at which half of all the DNA molecules in a sample denature; it varies according to the length of the molecule, the base-pair sequence, and the concentration of the genetic material in solution (1). Degradation refers to any decrease in quality of the polymer, including breaks between individual nucleotides in dsDNA, damaged bases, and fractures in the phosphate backbone. ssDNA is prone to degradation, as it is a less stable molecule than dsDNA. This paper defines "degradation" as breaks in the backbone of a DNA molecule that completely sever either dsDNA or ssDNA into many pieces. Degradation is usually undesirable in experiments that require intact DNA to be available for study; it may be facilitated by excessive heating of the molecule, and hence, it can be minimized by agents that raise the T_m and prevent denaturation.

The progressive degradation of DNA can be visualized by gel electrophoresis. DNA samples, stained with chemical agents that intercalate between paired nucleotides, are placed into a gel matrix through which an electric current is run. As DNA moves along the current towards the anode, the stain travels with the genetic material and marks its position after the process is over. The final position of the DNA molecule depends on its size, as smaller fragments move farther along the gel than larger ones. This analysis can help determine the level of degradation of DNA molecules, since the more degraded a sample is, the more the phosphate backbone has been cleaved and the smaller the individual fragments of DNA are; thus degraded sample material moves farther down the gel than intact DNA.

DNA is an acid, and after its dissociation in water, the polymeric molecule loses protons from phosphate groups in its sugar-phosphate backbone and becomes negatively charged. Various studies have linked this property of DNA to electrostatic interactions with metal cations. For example, Mg^{2+} has been shown to be important for stabilizing DNA in the human body and in aiding crucial enzymatic interactions with genetic material (2, 3). Duguid *et al.* suggest that magnesium interacts with the phosphate group in the DNA backbone and neutralizes the negative charges of each phosphate group (4). These charges would normally repel each other, thus putting tension on the polymer; however, since magnesium counteracts them, there are more

base-stacking interactions within the molecule (5). Increased base stacking is associated with a higher T_m of DNA, improving DNA stability and resistance to degradation (6).

This effect has been proven to be concentration-dependent. Different studies (7, 8) disagree about the effects of specific concentrations, but there appears to be a general pattern to these effects. In lower concentrations, magnesium ions complexed with DNA can increase the T_m, and thus the stability of the polymer; however, in high concentrations, magnesium salts will lower the T_m and facilitate the denaturation and degradation of the molecule. Effects of calcium, copper, and zinc have also been analyzed; calcium was shown to have a similar effect as magnesium in increasing DNA stability, while zinc inhibited the annealing, or re-joining, of single-stranded DNA molecules into double-stranded DNA, especially in sections with many repeats of guanine and cytosine (8, 9, 10). Clearly, different cations can have unique and interesting effects on DNA, which can be utilized in processes involving denaturation – magnesium is already used in PCR, for example, as a cofactor for Taq polymerase to stabilize the DNA strand to which the polymerase adheres (11). The question arises – what if other cations can be successfully used for such purposes? For example, can the effects of nickel, ammonium, and lithium cations in solutions containing DNA be compared to those of magnesium?

If so, perhaps these ions could be useful in developing new research techniques involving the degradation and preservation of DNA. Assays involving different cations can allow for flexibility in research protocols when studies are constrained by the materials or types of processes that are available to manipulate certain qualities of DNA. This investigation will focus on using cations to tweak the T_m of DNA and therefore achieve preservation or degradation of the sample. Since the chemical interactions between cations, such as magnesium, and the DNA molecule are mostly based on the charges of the two, ions similar to magnesium are expected to have analogous effects on DNA. Lithium was chosen for this investigation because it has a small ionic radius similar in size to that of magnesium; the ionic radius of Li^+ is 76 pm, and that of Mg^{2+} is 72 pm (12, 13). It is therefore expected that lithium can form bonds with a strength analogous to those that magnesium produces with DNA. However, the lithium cation is monovalent, compared to magnesium's divalent nature, and is therefore only electrostatically attracted to one phosphate group, as opposed to two. Ammonium ions also have a single positive charge, as lithium does, but otherwise bear little to no resemblance to magnesium because they are molecules and not elements, which may give ammonium ions unique effects that single-atom cations do not exhibit. The Ni^{2+} ion has a similar ionic radius to magnesium (69 pm; 14) and thus will also have bonds with a similar strength to magnesium; in addition, Ni^{2+} is also divalent like magnesium. Despite this similarity, nickel is a transition metal, which may grant it very different properties from Mg^{2+}. Hence, the hypothesized general effect is that Li^+, NH_4^+, and Ni^{2+} will all stabilize the polymer and raise its T_m, as magnesium does, by neutralizing the negative charges of the DNA's phosphate groups. However, the extent to which these cations will do so and whether any effects exclusive to each cation will manifest remain to be investigated. As these substances will be part of an ionic compound that is dissolved in water, anions will also be present in the medium; the anions are not expected to stabilize the molecule since the negative charges of the particles would repel them from DNA's electronegative phosphate groups. To verify this, magnesium sulfate is included in the study in order to compare its effects to the traditionally used magnesium chloride and to ensure that the anions did not interact with the DNA samples, since one of the salts used in the study was $(NH_4)_2SO_4$.

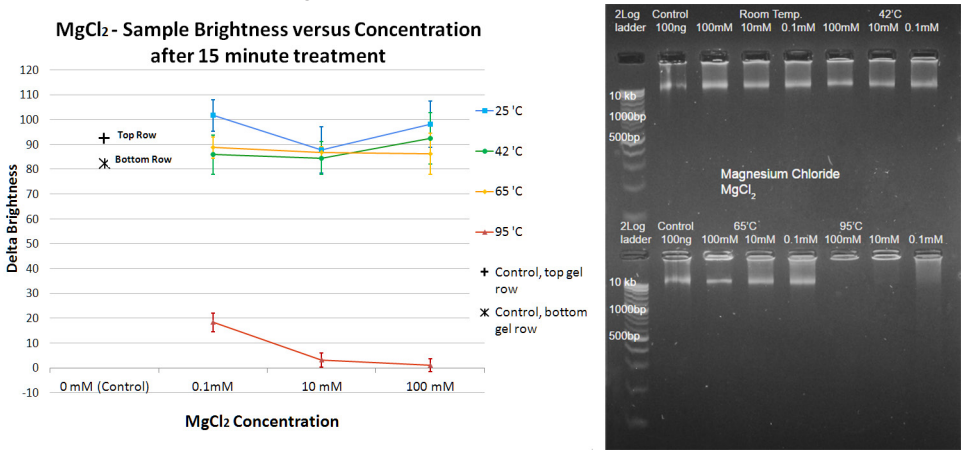

Figure 1. Delta brightness analysis and original gel image for $MgCl_2$ tests. Negative values on brightness scale is included to reflect standard deviation data.

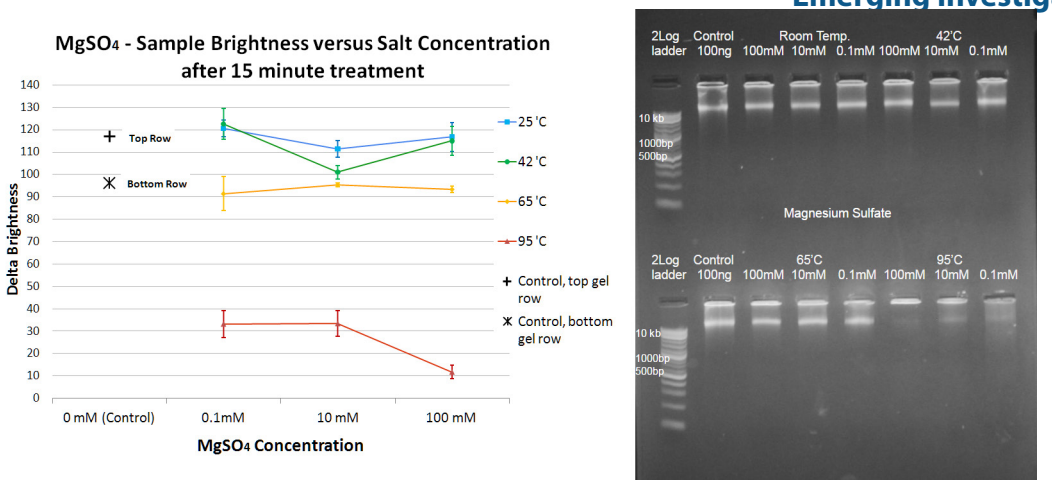

Figure 2. Delta brightness analysis and original gel image for $MgSO_4$ tests.

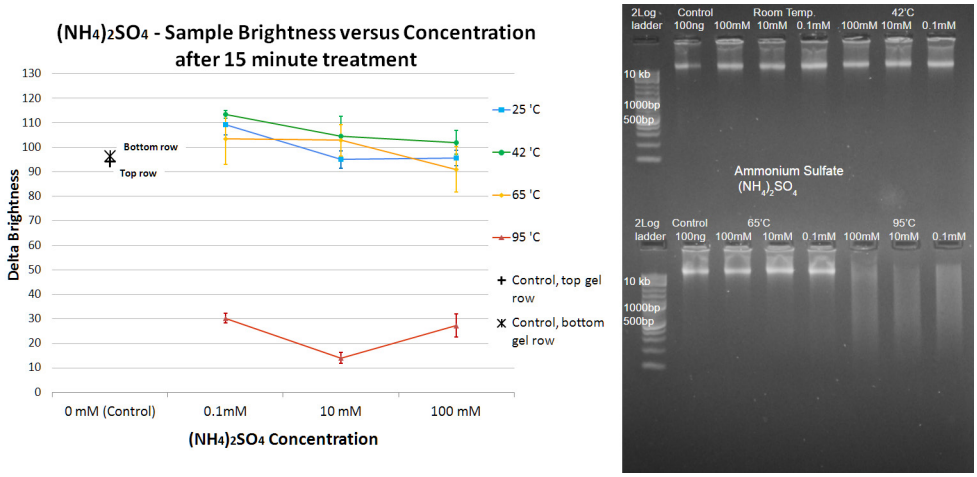

Figure 3. Delta brightness analysis and original gel image for $(NH_4)_2SO_4$ tests.

Results

In initial tests, portions of 100 ng/µl DNA were suspended at identical concentrations in solutions of magnesium chloride, magnesium sulfate, ammonium sulfate, lithium chloride, and nickel chloride. Each salt was mixed in three different concentrations, including 100 mM, 10 mM, and 0.1 mM. Then, DNA in each salt concentration was incubated at different temperatures (25°C, 42°C, 65°C, and 95°C) for 15 minutes. The products were then loaded onto an agarose gel and allowed to run in a gel electrophoresis machine, along with a control sample of untreated 100 ng/µl DNA at the same concentration in order to compare the quality of treated DNA to intact genomic material. The gels were then photographed on a UV transilluminator. The obtained images were analyzed with ImageJ software to determine the brightness of the final DNA product in the gel picture by subtracting the background gel's brightness from the control lane brightness and from each sample lane's brightness (see Methods for a more detailed explanation).

Mg^{2+} in both of its salt forms and NH_4^+ behaved similarly, with discrepancies only visible on the gel and not evident in the scope of the quantitative analysis (**Figures 1–3**). The general pattern was thus: all DNA samples remained intact for all salt concentrations at 25°C, 42°C, and 65°C, as the sample delta brightness was within one standard deviation of or significantly brighter than the average control brightness value; this indicates that the treated DNA was not degraded by the applied treatments. All samples were also, to various degrees, destroyed at 95°C, as the delta brightness was very low. Judging by a strictly qualitative analysis, replacing the Cl^- ion bonded with Mg^{2+} with SO_4^{2-} had a minimal effect on DNA quality because both molecules' negative charges are repelled by similar negative charges in the DNA backbone. Comparing the last 3 lanes of the bottom rows on gels displayed in **Figures 1 and 2**, it is evident that magnesium sulfate preserves slightly more of the 10-kb DNA sample than magnesium chloride at 95°C. Ammonium sulfate, rather than displaying the banded pattern of magnesium sulfate, exhibits an even smearing

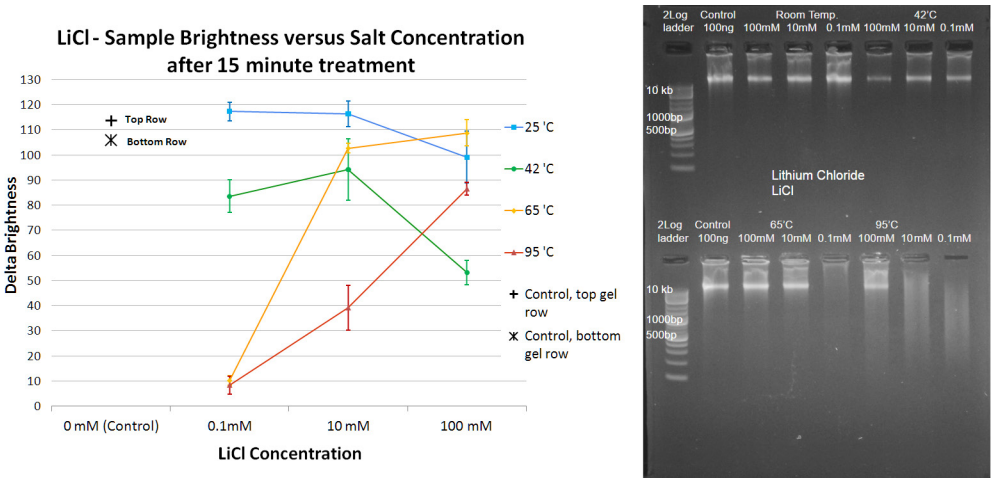

Figure 4. Delta brightness analysis and original gel image for LiCl tests.

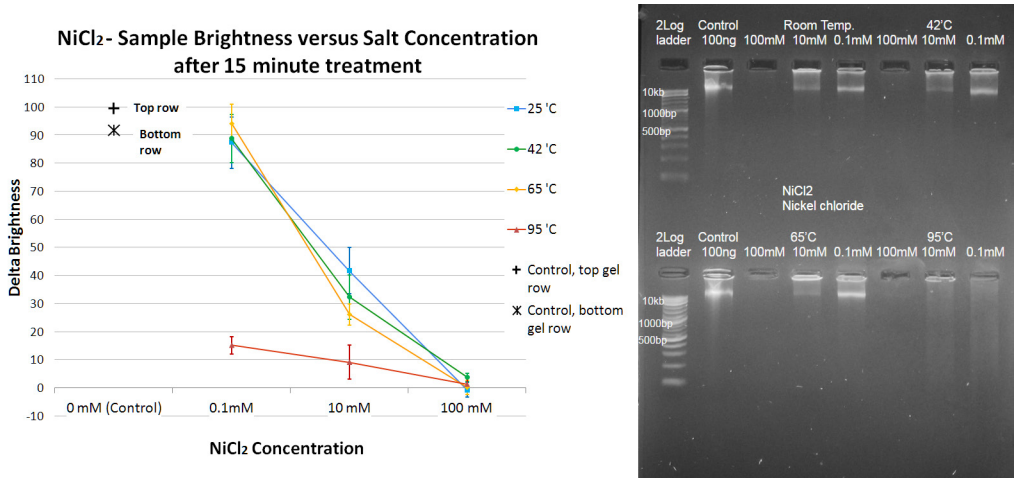

Figure 5. Delta brightness analysis and original gel image for $LiCl_2$ tests.

pattern (**Figure 3**); however, such information cannot be deduced from the values obtained by delta brightness analysis. A more comprehensive comparison can be seen in **Figure 6**, which displays the brightness of each lane along its length; as the brightness changes along each lane, so does the DNA concentration. Downward peaks represent brighter regions of each lane where DNA is more concentrated. On these images, the difference can be clearly seen between each type of treatment at 95°C. $MgCl_2$ has a single dip followed by a smear that begins at the well and is evenly spread down the lane; $MgSO_4$ peak patterns indicate a band with a small intermediate peak inside the smear, while $(NH_4)_2SO_4$ results indicate only slight smears that are uniformly weaker and which start significantly farther away from the well than those of $MgCl_2$.

Interestingly, when Ni^{2+} was applied at 100 mM and 10 mM, a distinct difference in the sample quality was observed as the samples, normally intact when treated with other salts, were destroyed (**Figure 5**). Lithium chloride displayed some relatively peculiar results as well, as it seemed to facilitate degradation at 100 mM in 42°C and destruction at 0.1 mM in 65°C (**Figure 4**). However, Li^+ also demonstrated the ability to preserve DNA at 100 mM in 95°C, conditions at which other salts did not effectively maintain a good sample quality. Additional tests were performed to corroborate initial trials, including a test of all salts at 95°C, as well as an additional test for lithium ions at 65°C (**Figure 7**). All aforementioned results were clearly repeated.

To investigate the preservative effects of lithium chloride, a DNA sample was first dissolved in 100 mM Li^+, then incubated at 95°C. The tested DNA was then cleaned through a column filter to remove all Li^+ ions and once again heated at 95°C for 20 minutes. A portion of the sample material was preserved at each step. The results show that lithium chloride preserved DNA heated to 95°C and evidently did not affect the sample quality – the treated material again behaves like normal, untreated DNA during heating once the lithium ions were removed (**Figure 8**).

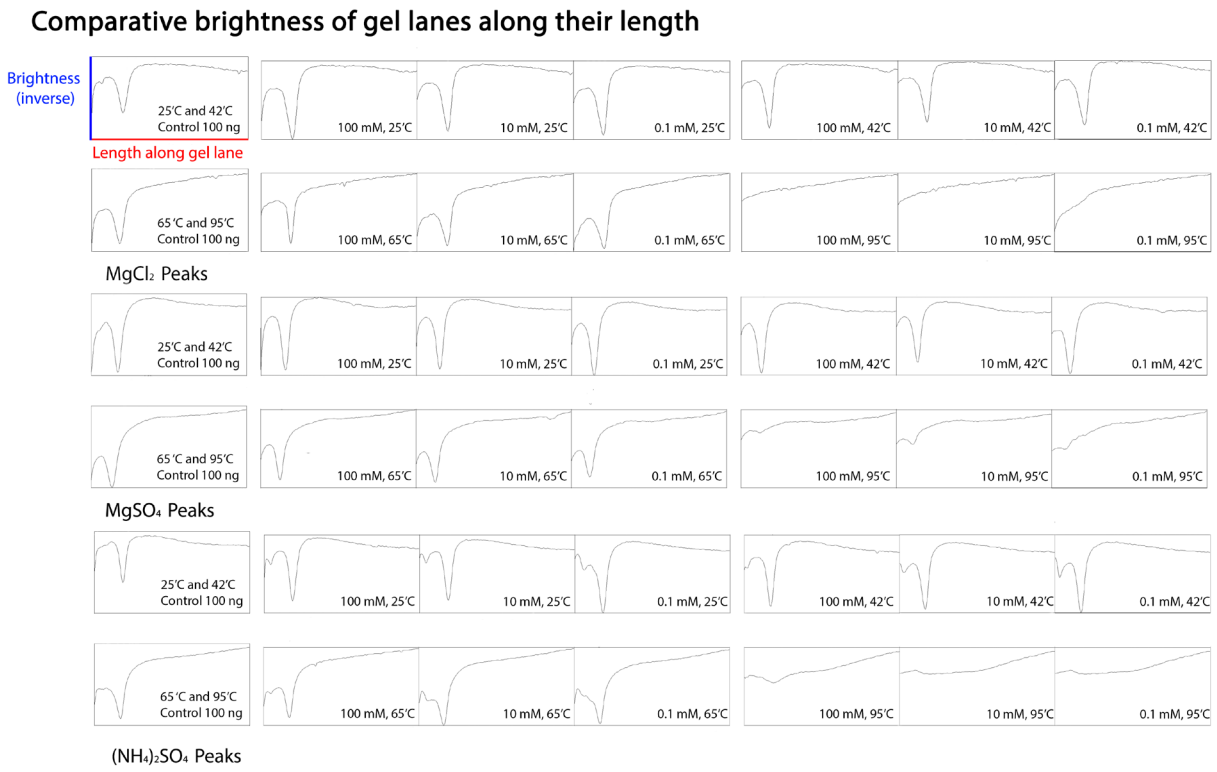

Figure 6. Brightness peaks for $MgCl_2$, $MgSO_4$, and $(NH_4)_2SO_4$ treatments. Results are arranged in the order each sample appeared on original gel images. Axes labeled on the first graph apply to all graphs.

Discussion

Magnesium chloride, magnesium sulfate, and ammonium sulfate displayed similar preservation patterns at all concentrations. All DNA samples incubated at temperatures below 95°C were preserved intact, and all samples were deemed severely degraded or destroyed at 95°C. However, it is important to note that the technique used in this study to quantify the qualitative difference between samples proved to be poorly representative of variation in quality between highly degraded samples. As seen in **Figure 6**, where magnesium chloride and ammonium sulfate are compared, it is evident that the samples degraded in noticeably different fashions at 95°C; however, the quantitative analysis of these data does not reflect this difference adequately. The comparative peak analysis displays some of this difference much more accurately; however, the reason for this difference is unclear, especially the increased appearance of banding patterns in $MgSO_4$ over $MgCl_2$. Such behavior was repeated in **Figure 8**, as seen in the last six lanes of the second row on the gel, and thus it was not the result of a mistake caused by variation in how the experiments were prepared. However, such a difference should not theoretically exist because anions would not interact with the negatively charged phosphate backbone of DNA and thus would have no effect on its stability. Although more research is needed to establish the reason for this difference, the disparity in results is so slight that the three treatment types do not give significant advantages over each other, as treatment with $MgSO_4$ did not preserve a distinctly greater amount of DNA than a treatment with $MgCl_2$. The application of $(NH_4)_2SO_4$ perhaps preserved even less intact DNA than the $MgCl_2$ treatment, as evidenced by a decreased amount of DNA fragments longer than 10 kb as compared to the former, as seen in both **Figures 1 and 3** and the last three lanes of the first row in **Figure 8**. Hence, when considering the treatments for use in experimental protocols, no significant advantage is gained from using $MgSO_4$ or $(NH_4)_2SO_4$ over $MgCl_2$.

Nickel chloride and lithium chloride, on the other hand, displayed very interesting results, and appeared to have almost completely opposite effects on DNA samples. Samples treated with high concentrations of nickel chloride did not display any distinct banding patterns on the gel, even at the relatively low temperature of 25°C, which indicates an extensive breakdown of the polymer, perhaps through the breaking of the dsDNA sample into shorter oglionucleotides or single nucleotides. While the exact mechanism for the degradation of the sample is not deducible through the methods used in this paper, part of the effect may be explained by nickel's reported affinity for aggregating around base pairs rather than the phosphate backbone of the DNA molecule. Duguid

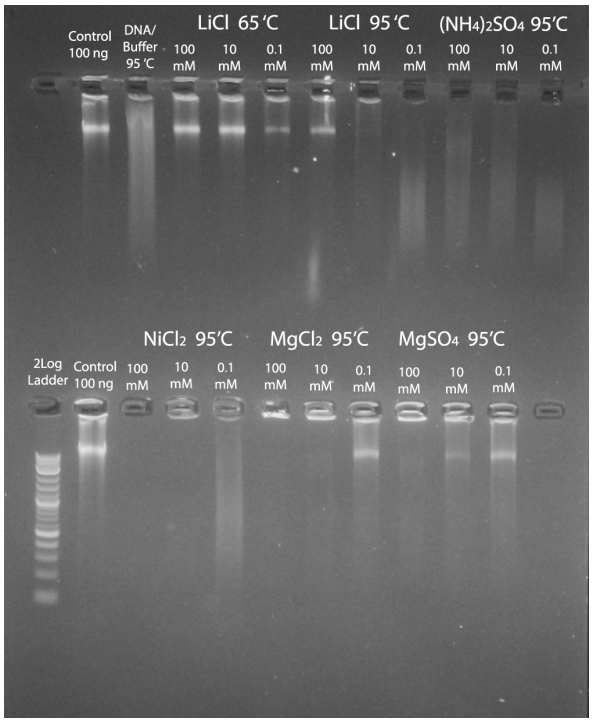

Figure 7. Comparative tests of all salts at 95°C and of an additional test of lithium treatment at 65°C.

et al. (1995) suggest that magnesium interacts with the phosphate group in the DNA backbone – thus the ions have a stabilizing effect on the molecule by neutralizing the negative charges of each phosphate group. Nickel, however, interacts with the DNA nucleotides; Duguid *et al.*, in their study of other metal cations, indicate Ni^{2+} interacts especially readily with the N7 atom of guanine. This results in the disruption of hydrogen bonds within the molecule and its eventual denaturation (4). This particular property may work in tandem with other effects, possibly ones that disrupt the phosphate backbone of the polymer as opposed to altering the T_m of the material, which, when combined, led to the sample degradation that was detected by gel electrophoresis analyses in this study. This may be one of the processes that accounts for nickel's carcinogenic properties, as well as why it has been found to interfere with DNA repair and facilitate oxidative stress (16). A similar affinity for guanine was reported for the aforementioned zinc (10), and Duguid *et al.* indicate other transition metals may also act in a similar manner. Such effects would need to be investigated further to be confirmed.

Lithium chloride is a more ambivalent cation in its effects. Results show degradation uncharacteristic for Mg^{2+} in 100 mM, 42°C and 0.1 mM, 65°C samples. Conspicuously, lithium ions at 100 mM preserved a sample relatively intact at 95°C, unlike the other two salts, signifying that Li^+ raises the T_m of DNA in the sample. This may be a result of Li^+ neutralizing the negative charges

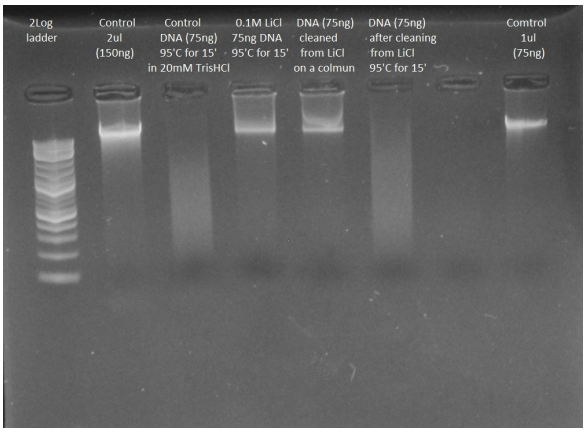

Figure 8. Additional testing to display the effects of lithium treatment at 95° C.

in the DNA backbone, as evidenced by the effect it has on DNA molecules that pass through nanopores. As nanopore-based protocols are dependent on an electric current to advance molecules through the nanopores for detection, DNA molecules usually pass through quickly due to their relatively high electronegativity; Kowalczyk *et al.* found that, with the addition of lithium ions to the experimental media, DNA traverses the nanopore much more slowly, indicating that the negative charges of the polymer were neutralized (17). The strong electronegativity decrease resulting from lithium treatment did not have a visible effect on gel electrophoresis analyses, as results show that intact treated samples did not move any slower through the gel than the untreated control. The cation's apparent degradative ability is perhaps explained in a study by Dong *et al.*, which indicates that lithium ions, as well as ammonium ions, bind effectively to lengthy tracts of repeating adenine bases in the minor grooves of the DNA helix (18). This suggests that Li^+, as well as NH_4^+, interact with DNA nucleotides and could disrupt the hydrogen bonds between them, as proposed in **Figure 9**. The aberrant results obtained for lithium at 42°C and 65°C and the aforementioned smearing of ammonium samples at 95°C may be byproducts of the cations separating nucleotides and hence denaturing the polymer by a mechanism similar to the one hypothesized for nickel. Despite this behavior, DNA treated with 100 mM Li^+ at 95°C behaved exactly the same as intact, untreated DNA after all lithium ions were removed from it, which indicates that the bases were not attacked sufficiently to noticeably degrade the sample. This suggests that the preservative effects of lithium ions are much stronger under certain conditions than their degradative properties.

A possible source of additional degradation of the polymer could have been the pH of the TE buffer used to store and process the DNA samples. At pH values between 9 and 5, the T_m changes little and the stability of the polymer is generally unaffected; however, at pH

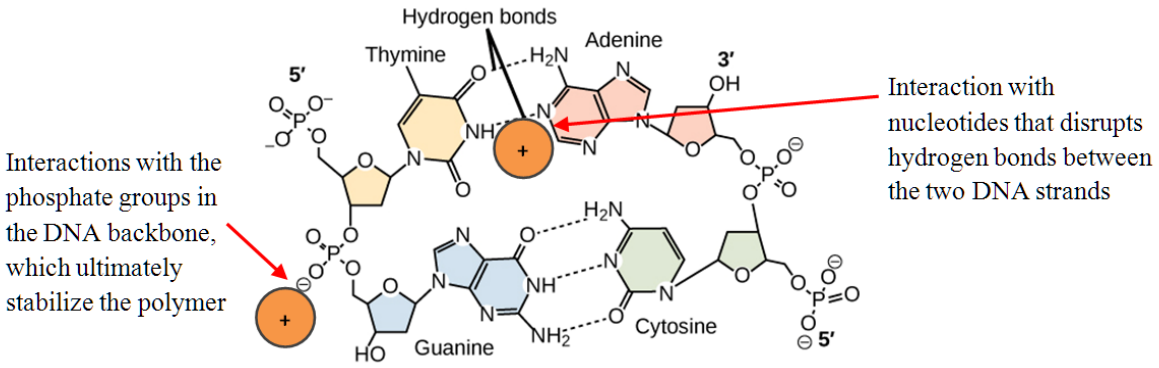

Figure 9. Proposed interaction sites of cations, either Li+ or NH_4^+(19).

values outside that range, the bonds between bases can be disrupted. At exceedingly high pH values, guanine and thymine can become deprotonated, losing a hydrogen atom involved in the hydrogen bonding between complimentary base pairs, which would be a source of significant instability within the molecule that may cause the polymer to denature; at low pH values, cytosine and adenine become protonated, and the extra hydrogen also impedes bonding to complementary bases resulting in similar instability in the sample (20). The pH of the TE buffer used in the protocol was 8.0, so neither of the above described effects should have occurred since the buffer should generally neutralize any factors that affect pH, and hence, the pH within the buffer should not fluctuate outside the 5–9 pH range. An undesirable pH should not be a source of the different degradation patterns described above.

While magnesium chloride, magnesium sulfate, and ammonium sulfate do not offer any significant advantage over each other in preserving or degrading DNA, the implications for the results obtained from nickel chloride and lithium chloride samples are more extensive. Nickel as a DNA degrading agent is highly effective; at high concentrations its effects cannot be controlled. At lower concentrations, nickel ions could be used in protocols requiring the lowering of the melting temperature of DNA, an application which further tests could investigate. Lithium ions could be used for the effective preservation of DNA molecules in extreme conditions, such as when a sample must remain viable without adequate refrigeration in high-temperature environments. Since lithium ions exhibit different properties of conservation and degradation depending on concentration and temperature, lithium chloride can also be utilized to finely control the forces acting on a DNA molecule and thus transition from one effect the cations have to the other by simply adding or removing lithium ions from solution in the course of a protocol. Moreover, Li^+ can be used in protocols calling for a significantly reduced electronegativity of DNA molecules, such as in the aforementioned nanopore experiments (17), to allow for more efficient study of DNA molecules. To clarify how well lithium ions can preserve genetic material, further tests must be conducted for more prolonged periods of heat exposure, at different concentrations of Li^+, and at different temperatures. Further studies are needed to determine when lithium's degradative properties take precedence over its preservative processes. Tests that indicate whether lithium salts are capable of correctly preserving known base pair sequences are crucial to any further studies, as it is evident that Li^+ interacts with nucleotides and may therefore compromise the genetic code in ways that a simple gel electrophoresis analysis would not show, and if lithium ions significantly disrupt the sequence of the nucleotide bases, Li^+ may not be useful in scientific protocols requiring an accurate base pair sequence to be maintained.

Materials and Methods

DNA Sample Preparation

DNA was extracted from *Acheta domesticus* using the phenol-chloroform method as described in Davies *et al.* (15). Four separate samples were prepared, and a Nanodrop 1000 spectrophotometer was used to determine the sample with the most suitable concentration. The DNA was then cleaned twice according to the Zymo Research DNA Clean & Concentrator-5 protocol (21). The sample used for most of the experiments had a concentration of 100 ng/µl; for the lithium chloride additional testing procedure, a different, 150 ng/µl sample was used as a control, and was diluted to 75 ng/µl for the test itself. All samples, both control and leftover experimental samples, were stored at -20°C in TE buffer with a pH of 8.0 while not in use for the duration of the study.

Preparation of Solutions

The solid forms of $NiCl_2 \cdot 6H_2O$, $MgCl_2$, $(NH_4)_2SO_4$, LiCl, and $MgSO_4$ salts were mixed at 100 mM, 10 mM, and 0.1 mM in deionized water and stored at room temperature (25°C) for the duration of the study.

Treating DNA Samples

Three heating blocks were preset to 42°C, 65°C, and 95°C. A portion of the sample DNA was mixed with a certain ionic solution in a ratio of 4 µl of original DNA to 80 µl of solution. The resulting mixture was then divided into four separate Micro Test Tubes, with 21 µl of the mixture in each tube; two of the resulting four samples were covered with approximately three drops of mineral oil to prevent evaporation. The mineral-oil-covered samples were incubated at 65°C and 95°C. The third sample was placed at 42°C, and the fourth remained at 25°C. The four samples were incubated at their respective temperatures for 15 minutes, and then placed in 4°C ice to cool while a gel for gel electrophoresis was prepared.

Gel Electrophoresis

Standard 1.5% agarose gels in Tris-Borate-EDTA buffers were prepared to visualize all trials. A GelRed intercalating staining agent obtained from Biotium Inc. was used to allow the DNA samples to be identified under UV light by their distinctive glow. For each gel, the first well was filled with 2 µl of 2-Log DNA ladder from New England Biolabs (NEB), the second with 1 µl of the 100 ng/µl control DNA sample mixed with 2 µl of DNA loading dye from NEB. The rest of the wells were filled with 100 ng of the incubated sample DNA mixed with 2 µl of dye.

The gel was then allowed to run for 30 minutes at 100 Volts. Afterwards, it was removed from the gel electrophoresis machine and photographed using a UV transilluminator.

Analysis of results

The images obtained by photographing gels on the transilluminator were analyzed by finding the relative brightness of each individual DNA sample using ImageJ software. The brightness of the background area and of each sample was determined by selecting the respective area and averaging the red, green, and blue channels with the following formula (22):

$$V = \frac{R + G + B}{3}$$

In this equation, R, G, and B represent the red, green, and blue channel values, respectively, and V represents the obtained value. The program automatically calculated this value through the "Analyze" command. Each area was analyzed five times, and the values obtained by each analysis averaged. A standard deviation value was calculated from the five analyses. The average value obtained from the background analyses was subtracted from the average values obtained for each sample, and the resulting delta brightness values were plotted on **Figures 1–5** along with the standard deviation data mentioned above.

Brightness peaks analysis

The gel images obtained for $MgCl_2$, $MgSO_4$, and $(NH_4)_2SO_4$ were analyzed using the gel analysis process offered by ImageJ software (23). Sections of equal area enclosing each DNA band except for the ladder were marked out on gel images and indicated as gel lanes to be analyzed for brightness. An automated algorithm then evaluated the brightness along each lane and displayed the results in a graph format. Each dip in the graph indicated a region brighter than the background luminosity and thus graphically portrayed the location of peaks or brightness gradients along each gel lane for subsequent comparison among different treatment types. The graphs were arranged in order as each gel lane would appear on the original gel image and displayed in **Figure 6**.

Additional testing

Any additional testing was conducted with the same protocol as outlined above, except for the changes specifically listed in this section. Salts at all concentrations were tested at 95°C for 15 minutes along with a sample of LiCl tested at 65°C to confirm results obtained in initial trials; the resulting gel is displayed in **Figure 7**. To quantify the preservative effects of LiCl, a DNA sample was first dissolved in 100 mM LiCl, then incubated at 95°C. The DNA was then cleaned through a column filter to remove Li^+, and once again heated at 95°C for 20 minutes; each step, as well as 3 controls (one with a standard untreated DNA at 150 ng/µl, one with an untreated 75 ng/µl concentration sample, and a 75 ng/µl concentration sample heated at 95°C for 20 minutes), were loaded onto a gel along with the experimental samples for analysis. The gel results are shown in **Figure 8**.

References

1. DNA Melting Temperature. "Gene Synthesis Biotechnology Service Provider DNA Melting Temperature Comments." Entelechon, n.d. Web. 01 Nov. 2015.
2. Luscombe, Nicholas M., Roman A. Laskowski, and Janet M. Thornton. "Amino Acid–base Interactions: A Three-Dimensional Analysis of protein–DNA Interactions at an Atomic Level." *Nucleic Acids Research* 29.13 (2001): 2860–2874. Web. 29 Aug 2015.
3. Hartwig, A. "Role of Magnesium in Genomic Stability." *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* Volume 475.1-2 (2001):

113-21. Web. 30 Aug. 2015.

4. Duguid, J. G., V. A. Bloomfield, J. M. Benevides, and G. J. Thomas. "Raman Spectroscopy of DNA-metal Complexes. II. The Thermal Denaturation of DNA in the Presence of Sr^{2+}, Ba^{2+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, and Cd^{2+}." *Biophysical Journal* 69.6 (1995): 2623–2641. National Center for Biotechnology Information. U.S. National Library of Medicine, n.d. Web. 1 Aug. 2015.
5. Brown, Tom, and Tom Brown, Jr. "DNA Duplex Stability." Nucleic Acids Book. N.p.: n.p., n.d. N. pag. ATDBio. ATDBio Ltd. Web. 05 Dec. 2015.
6. Yakovchuk, Peter, Ekaterina Protozanova, and Maxim D. Frank-Kamenetskii. "Base-stacking and Base-pairing Contributions into Thermal Stability of the DNA Double Helix." *Nucleic Acids Research* 34.2 (2006): 564-74. Oxford University Press. Web. 5 Dec. 2015.
7. Sorokin, V., V. Valeev, and E. Usenko. "Mg^{2+} and Ca^{2+} Ion Effect on DNA Thermal Stability in the Field of Formation of Polynucleotide Metallic Forms." Reports of the National Academy of Sciences of Ukraine 4 (2009): 183-88. *Presidium of the National Academy of Sciences of Ukraine*. Web. 29 Aug. 2015.
8. Schank, George. "The Effect of Metal Ions of Varying Sizes and Concentrations on DNA Melting Temperature." McKendree University, 2007. Web. 1 Aug. 2015.
9. Blagoĭ, IuP, VA Sorokin, VA Valeev, and GO Gladchenko. "Effect of Divalent Copper Ions on Heat Denaturation of DNA." Molecular Biology (1978): 795-805. National Center for Biotechnology Information. U.S. National Library of Medicine. Web. 29 Aug. 2015.
10. Lu, Shunwen. "Zn^{2+} Blocks Annealing of Complementary Single-Stranded DNA in Sequence-Selective Manner." *Nature Publishing Group*, 26 June 2014. Web. Accessed 1 Aug. 2015.
11. Grunenwald, Haiying. "Optimization of Polymerase Chain Reactions." PCR Protocols. Ed. John M. S. Bartlett and David Stirling. Second ed. Vol. 226. Totowa, NJ: Humana, 2003. 92. Ergonomics: University of California. Regents of the University of California. Web. 5 Dec. 2015.
12. "Radii for Li." Database of Ionic Radii. Atomistic Simulation Group, n.d. Web. 21 July 2016. <http://abulafia.mt.ic.ac.uk/shannon/radius.php?Element=Li>.
13. "Radii for Mg." Database of Ionic Radii. Atomistic Simulation Group, n.d. Web. 21 July 2016. <http://abulafia.mt.ic.ac.uk/shannon/radius.php?Element=Mg>.
14. "Radii for Ni." Database of Ionic Radii. Atomistic Simulation Group, n.d. Web. 21 July 2016. <http://abulafia.mt.ic.ac.uk/shannon/radius.php?Element=Ni>
15. Davies SW, *et al.* (2012). "Novel polymorphic microsatellite markers for population genetics of the endangered Caribbean star coral, *Montastraea faveolata" Marine Biodiversity* 43 (2): 167–172.
16. Cameron, Keyuna S., Virginia Buchner, and Paul B. Tchounwou. "Exploring the Molecular Mechanisms of Nickel-Induced Genotoxicity and Carcinogenicity: A Literature Review." *Reviews on environmental health* 26.2 (2011): 81–92. *National Center for Biotechnology Information*. U.S. National Library of Medicine, n.d. Web. 1 Aug. 2015. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3172618/?report=classic>.
17. Kowalczyk, Stefan W., David B. Wells, Aleksei Aksimentiev, and Cees Dekker. "Slowing down DNA Translocation through a Nanopore in Lithium Chloride." *National Center for Biotechnology Information.* U.S. National Library of Medicine, 27 Jan. 2012. Web. 1 Aug. 2015.
18. Dong, Qian, Earle Stellwagen, and Nancy C. Stellwagen. "Monovalent Cation Binding in the Minor Groove of DNA A-tracts." *National Center for Biotechnology Information*. U.S. National Library of Medicine, 10 Feb. 2009. Web. 1 Aug. 2015. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2662744/>.
19. Image modified from: *Figure 9.4.* Digital image. *Principles of Biology*. Lumen Learning, n.d. Web. 15 Nov. 2015.
20. Doktycz, Mitchel J. "Nucleic Acids: Thermal Stability and Denaturation." Encyclopaedia Of Life Science (1997): 6. Oak Ridge National Laboratory. Oak Ridge National Laboratory. Web. 22 July 2016. <http://web.ornl.gov/~webworks/cpr/misc/107675_.pdf>.
21. "Instruction Manual for DNA Clean & Concentrator™-5 Catalog." (n.d.): 4. *Zymo Research*. ZYMO RESEARCH CORP. Web. 27 Sept. 2015. <http://www.zymoresearch.com/downloads/dl/file/id/35/d4003i.pdf>.
22. Ferreira, Tiago, and Wayne Rasband. "Analyze." *ImageJ User Guide*. National Institutes of Health, n.d. Web. 1 Sept. 2015. <http://rsbweb.nih.gov/ij/docs/guide/146-30.html>.
23. Miller, Luke. "Analyzing Gels and Western Blots with ImageJ." Lukemillerorg RSS. Luke Miller, 04 Nov. 2010. Web. 21 July 2016. <http://lukemiller.org/index.php/2010/11/analyzing-gels-and-western-blots-with-image-j/>