NUCLEIC ACID CATALYSTS: COMPARING THE MECHANISMS OF DNA AND RNA ENZYMES

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ABSTRACT

Based on previous research on DNA enzyme catalysis, it has been shown that divalent ions are effective in aiding in the cleavage of RNA substrate by the 10-23 DNA enzyme. In previous work by other laboratories, the 10-23 DNA enzyme was isolated by *in vitro* evolution and has been shown to cleave RNA effectively. It does this by binding to the RNA substrate via Watson-Crick base-pairing and, with cofactors, deprotonates the 2'-hydroxyl group on the RNA sugar. This experiment was designed to test the efficiency of monovalent ions as cofactors in comparison to divalent ions. The monovalent ions tested were lithium, ammonium, and potassium, compared against the divalent positive control of magnesium. These three monovalent ions were chosen based on several chemical properties, including ionic radius and metallic character, in order to determine if these traits had an impact on the function of the 10-23 DNA enzyme. The monovalent ions were used in higher concentrations than the divalent ion because past research demonstrated this was necessary to accommodate for the difference in valence shell structure. After the reactions ran to completion, polyacrylamide gel electrophoresis (PAGE) was used to interpret the results. After all the gels were analyzed, they showed that monovalent ions, as compared to divalent ions, are minimally effective at RNA cleavage. In light of the effectiveness of monovalent cations serving as catalytic cofactors for ribozymes, it seems that divalent cations play a more significant role stabilizing the DNA enzyme's catalytic conformation, and therefore these cations' function cannot be replaced by monovalent ions. This expands our understanding of the catalytic mechanism involved in the 10-23 DNA enzyme's cleavage of RNA substrate.

INTRODUCTION

The 10-23 DNA Enzyme

Molecular biology is founded on transcription of RNA from a DNA template and subsequent translation of RNA sequence into protein [1]. For many years, RNA was assumed to be solely used as a nucleic acid used to code for proteins ensuring an organism's survival. However, this changed upon the discovery of RNA enzymes, or ribozymes, which are capable of catalyzing chemical reactions [2], even though it was previously thought that protein enzymes were the only organic molecules with such capabilities. Many natural ribozymes also exhibit the ability to self-cleave, cleave other RNA molecules site-specifically, or even catalyze ligation reactions. RNA portions of ribosomes are also responsible for catalysis of peptide bond formation, indicating nucleic acids can play important catalytic roles [3].

Experimentation on RNA viruses caused scientists to question whether RNA is the only nucleic acid that can function as an organic catalyst. As a result, research began to experiment with DNA and other biopolymers, with the aim of creating a catalyst with a more stable structure than those of the ribozymes, while at the same time creating a nucleic acid catalyst that would last longer *in vivo*. By accomplishing this aim, valuable insights into the mechanisms of nucleic acid catalysts would be gained as well [4]. This goal was finally achieved in 1993 when Ronald Breaker and Gerald Joyce, using *in vitro* evolution, succeeded in creating a DNA enzyme that can effectively cleave RNA [5].
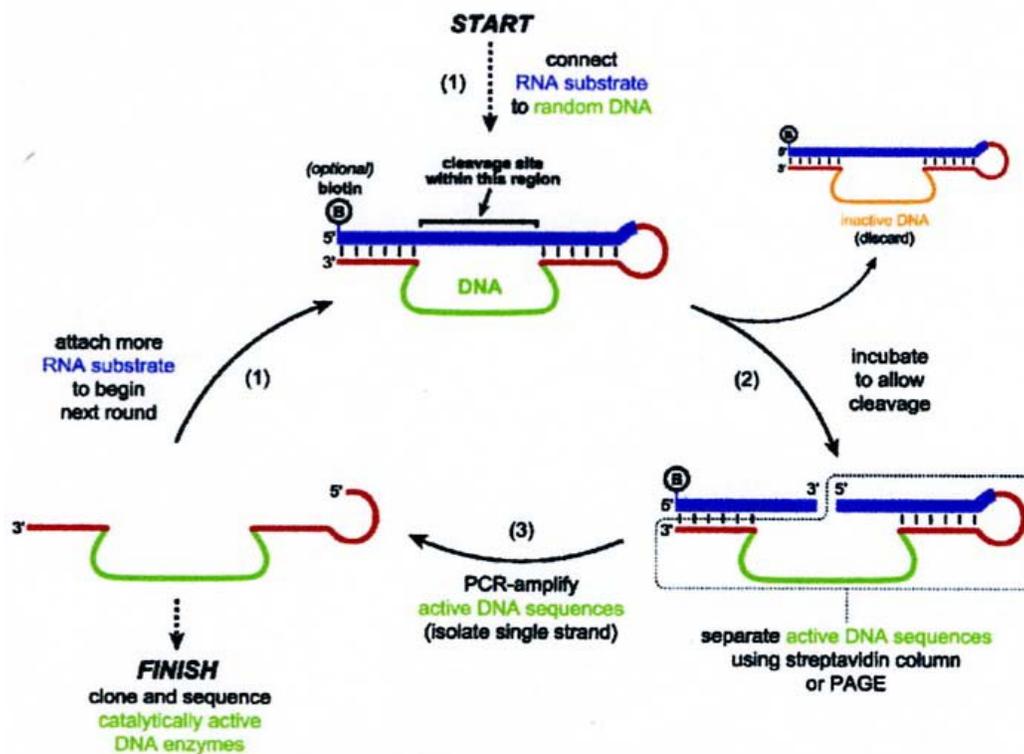
Figure 1 – Method of *in vitro* evolution and preparation of DNA enzyme [6]

The *in vitro* evolution process is designed to mimic Darwinian evolution. By using in vitro evolution, as shown in Figure 1, Santoro and Joyce isolated the 10-23 DNA enzyme, which is more effective at cleaving RNA than any other DNA enzyme [7][5]. The preparation of the DNA enzyme library is done by creating random oligonucleotide strands. The starting pool of DNA molecules contains a large number of distinct molecules. The differences in the molecules cause some to function as enzymes, which can cleave RNA. The strands are then added to RNA substrate. Active DNA that cleaves the RNA is isolated and identified by gel electrophoresis. Gel electrophoresis is described in detail later in the paper. Any DNA that cleaves RNA is retained and amplified by a polymerase chain reaction (PCR), while other DNA is discarded [6]. PCR is a process to make many copies of a strand of DNA by mimicking the replication mechanism found *in vivo*. The process is repeated with the active DNA for many rounds; 10-23 refers to the 23rd clone from the 10th round of selection.

Applications   Research on DNA enzymes has many applications in future biochemical research. One promising application of the 10-23 DNA enzyme is its use against RNA viruses such as HIV, SARS, and the human rhinovirus, a strain which causes the common cold. Potentially, DNAzymes could be constructed to specifically recognize these genomes and cleave viral RNA strands before they infect cells [8], [9], [10]. For instance, researchers from the Laboratory of Virology and Immunology in India have identified specific DNA enzyme cleavage sites on the single stranded portions of the HIV RNA strand indicating the feasibility of the 10-23 DNA enzyme cleaving HIV [11]. Previous researchers have even changed nucleotides in 10-23's catalytic core; such modifications increase 10-23's ability to splice many different RNA sequences. Greater applications of 10-23's catalytic core imply that it can target other RNA viruses even beyond known applications like degrading viral strains that cause the common cold [10]. Further research has shown that sections of the 10-23 DNA enzymes can be altered to increase its stability if it is introduced in living systems. Alteration of 10-23 DNA enzyme's arm length and catalytic core enables the enzyme to counter endonucleolytic degradation [10]. 10-23's increased stability implies greater survivability in complex biological systems. DNA enzymes created by in vitro evolution can not only react similarly as RNA enzymes but also last longer than even the best ribozymes in biological systems [6].

Comparison of Ribozymes with 10-23 DNA Enzyme
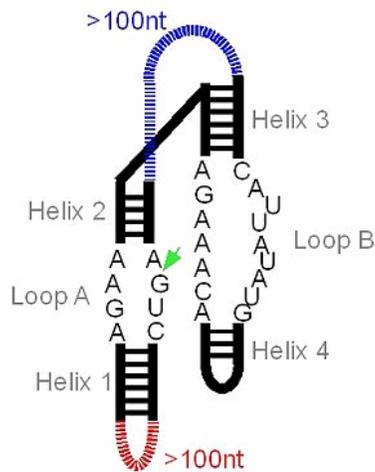
Figure 2.   The secondary structure of a hairpin ribozyme showing the four helices, two loops, and cleavage site at the arrow at loop A.   [12]
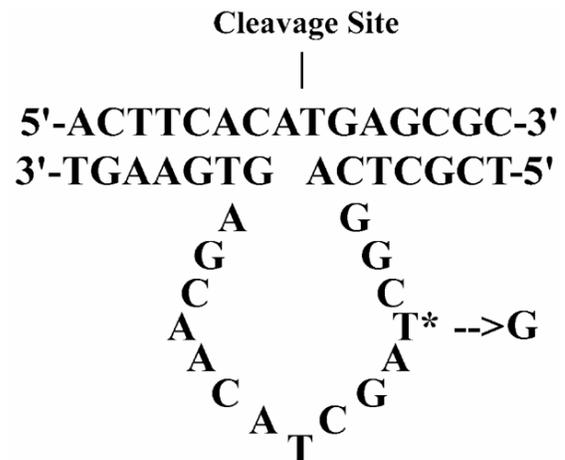
Figure 3.   The secondary structure of a 10-23 DNA enzyme showing the loop-like core and the cleavage site at the unpaired nucleotide. [13]

DNA enzymes work similarly to RNA enzymes. Both DNA enzymes and RNA enzymes cut RNA by cleaving the phosphodiester bond after recognition through Watson-Crick base pairing [14]. Certain structures are necessary for these enzymes to be catalytic. Scientists have previously studied three types of ribozymes that are similar to the 10-23 DNA enzmye: the hairpin ribozymes, VS ribozymes, and hammerhead ribozymes. As shown in Figure 2, the hairpin ribozymes are made up of four helices and two internal loops, A and B, with the cleavage

site at loop A. VS ribozymes are made up of 5 helices, with the cleavage site in the loop of helix I. The hammerhead ribozymes are made up of two hybridizing arms, a catalytic core, and a helix [12]. Conversely, as pictured in Figure 3, DNA enzymes have a loop-like reactive core with an unpaired nucleotide to aid in cleaving RNA [13].

DNA and RNA enzymes are both catalytic enzymes, however their different structures enable different capabilities. Ribozymes are required to fold into the correct three-dimensional structure to be catalytically active and have a high specificity for their target RNA. These qualities limit the ribozymes' catalytic capability. If the ribozyme is not folded correctly it will not be active and therefore not catalyze a reaction. With a high specificity, ribozymes only cleave certain RNA, thus making ribozymes difficult to use to target the other RNA outside of the ribozymes' natural field of specificity [15]. A study on the 10-23 DNA enzymes and hammerhead ribozymes found that, even though DNA enzymes need to fold into catalytic conformation, they are to an extent less specific about their cleavage sites, therefore making the DNA enzymes easier to manipulate *in vitro* [14].

Ribozymes and DNA enzymes both have the potential to turnover, which means they can cut more than one RNA strand in a reaction. Since they both have the advantage of cleaving more than one RNA substrate, the remaining factor is their rate of catalysis. In a study comparing 10-23 DNA enzymes with hammerhead ribozymes, kinetic analysis showed that the DNA enzyme has a 15-fold higher reaction rate under single turnover conditions compared with that of the fastest ribozyme [14].

The catalytic capability of DNA enzymes has proved to be a promising breakthrough; DNA enzymes have become a favorable candidate for use in catalytic reactions in place of ribozymes. DNA enzymes are known to be easier to synthesize and less susceptible to chemical and enzymatic degradation [16] which is ideal for *in vitro* applications. Continued research will lead to a greater understanding of this mechanism, which will allow for better design and manipulation of such DNA enzymes.

The Role of Divalent Ions in Nucleic Acid Enzyme Catalysis

In vivo, divalent metal ions are key to nucleic acid catalysis. In ribozymes, it was thought that a divalent ion catalytic cofactor, such as Mg^{2+}, was necessary to catalyze the cleavage reaction, since it stabilized the structure of the nucleic acid [17]. For example, scientists thought that divalent ions like Mg^{2+} in millimolar concentration ranges interacts with an Pro-R(P) oxygen on the phosphate of the hammerhead ribozyme to help initiate catalysis [18]. However, as scientists continued to search for the optimum environment for the function of the ribozymes, they decided to test the effectiveness of divalent metal ions other than Mg^{2+}. Research by Nowakowski, Shim, and their colleages revealed that divalent ions and a certain trivalent ion, cobalt (III) hexamine, interacts with a phosphate group for greater stability [6]. In 1998, Santoro and Joyce found that similar to the hammerhead ribozyme, the 10-23 DNA enzyme was active in the presence of divalent cations that included not only Mg^{2+}, but also Ca^{2+}, Mn^{2+}, and Pb^{2+} [7].
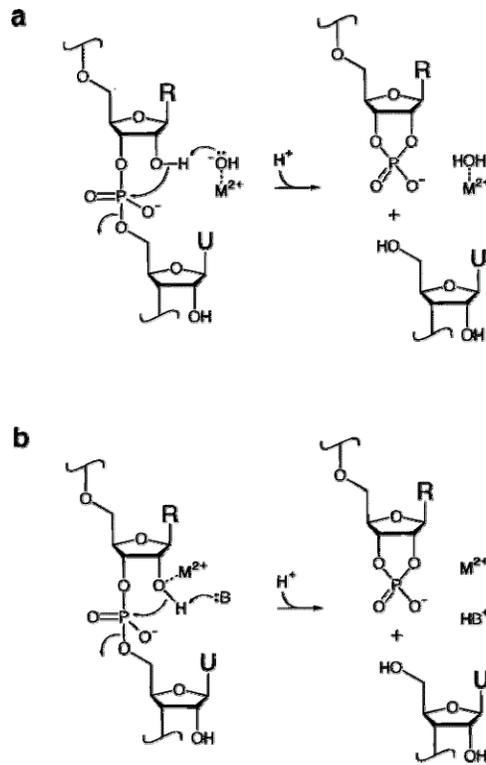
Figure 4: Two possible mechanisms in which a divalent metal ion serves as a catalytic cofactor for the 10-23 DNA enzyme's cleavage of RNA.

As pictured in Figure 4, the 10-23 DNA enzyme is proposed to induce RNA cleavage when a cation causes deprotonation of the 2' hydroxyl group on RNA. The nucleophilic cation is surrounded by water molecules and one of them loses a hydrogen to become a hydroxyl group. This causes the hydrogen in the 2' hydroxyl group to be pulled away, exposing a reactive oxygen. This oxygen then binds to the phosphate group, creating a 2'-3' cyclic phosphate bond. This process cleaves the bond to the 6' oxygen on the downstream sugar, which is broken and cleaves the RNA [7].

As scientists continued to search for the optimal conditions for the function of ribozymes, they discovered that these divalent cations might not in fact be needed. Murray, Seyhan, and their colleagues observed that various ribozymes were catalytically proficient in the presence of monovalent cations alone. They tested the effectiveness of the hammerhead, hairpin, and VS ribozymes in solutions of Li^+, Na^+, and NH_4^+, and discovered that if these ions are present in high concentrations, the rate of RNA cleavage the ions induced could be greater than or equal to that of solutions containing Mg^{2+} [17]. Given the successful tests of ammonium, these scientists also found that metal cations were not necessary to catalyze the reaction. In 2007, Fedoruk-Wyszomirska, Wyszka, and their colleagues proved that an environment containing both monovalent ions and high hydrostatic pressure, in the place of a divalent metal ion, can partially mimic the structural benefits of Mg^{2+} for ribozyme [19].

Purpose

In light of the proven effectiveness of monovalent ions in the function of various ribozymes, in this study monovalent ions were used to further understand how the 10-23 DNA enzyme functions in different environmental conditions. In particular, we wanted to test whether RNA and DNA enzymes had similar or different requirements for divalent metal ions. We decided to test the effectiveness of the 10-23 DNA enzyme in the presence of Li^+, K^+, and NH_4^+. These cations were chosen because they vary in structural and chemical properties. Since previous studies of RNA enzymes have demonstrated that ionic radii are log-linearly proportional to rate of reaction [20], Li^+ and K^+ were chosen for testing to see if the variation in their ionic radii (60 pm and 133 pm, respectively) [21], will differently influence the mechanism of the deprotonation reaction. In addition to these metal cations, the nonmetal polyatomic ion, NH_4^+, was chosen to test the effectiveness of the 10-23 DNA enzyme in the presence of nonmetallic cations.
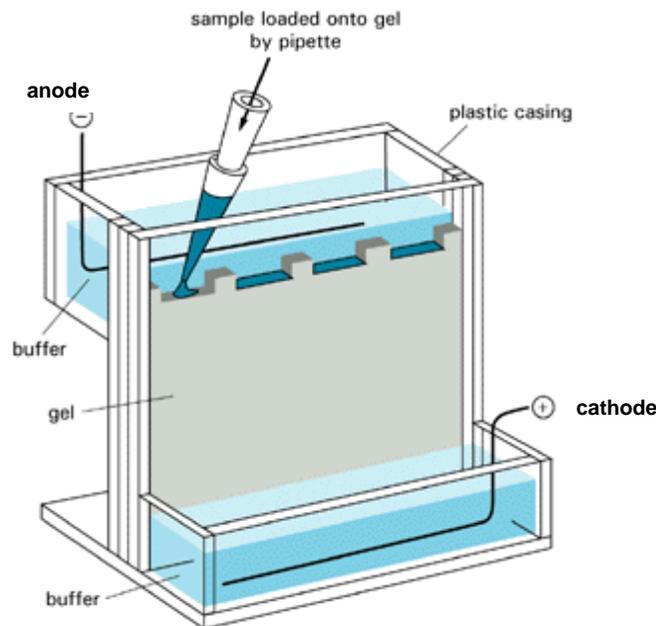
Figure 5: PAGE Gel [22]

To interpret the results, the samples were run through polyacrylamide gel electrophoresis (PAGE), shown in Figure 5. Gel electrophoresis is used to analyze fragments of nucleic acids. The gel separates the pieces of genetic material based on size and electric charge. When a gel is submerged in a buffer solution and an electric current is applied. The DNA and RNA are negatively charged and are pulled towards the cathode. The gel is a molecular sieve that divides the samples based on number of base pairs. PAGE is vertical rather than horizontal like some other gels. Gel electrophoresis with polyacrylamide was used because the experiment was involved with extremely small fragments of nucleic acids [1].

In order to ensure that divalent ions were removed from the monovalent reactions, ethylenediaminetetraacetate (EDTA) was added. EDTA was used at concentrations of 25 mM. EDTA is polydentate chelating agent with six coordinating atoms, allowing EDTA able to form strong bonds with divalent ions. This is useful in our reactions because the purpose is to show

that RNA cleavage can occur without the presence of divalent ions. It was used in reactions to bind to any divalent ions, such as Mg^{2+} or Ca^{2+}, and stop them from competing with the monovalent ion. EDTA is such a strong chelating agent because the four carboxyl groups and two amine groups have a great attraction for the divalent ion. EDTA is useful to remove heavy metal ions, which is why its found in many consumer products, such as soda, beer, and most cleaners, where it prevents metal ions from forming precipitates [21].
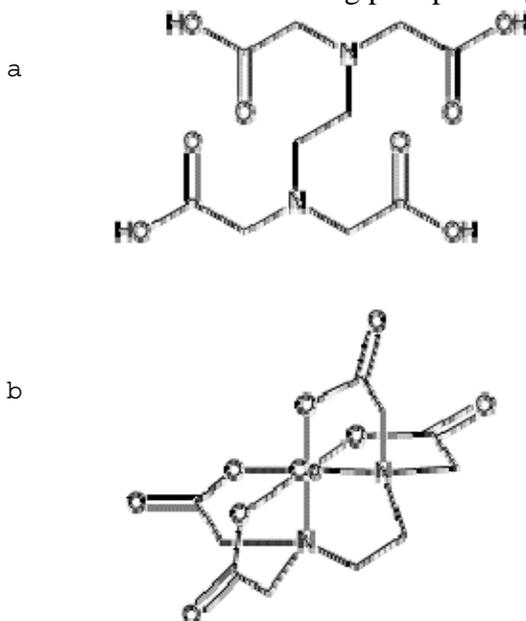
Figure 5: Depiction of EDTA structure alone (a) and when it binds to a divalent ion, such as Co^{2+} (b) [21]

This experiment affirmed that, compared to monovalent ions, divalent ions are indeed the more effective catalytic cofactors in the cleavage of RNA. If the first set of reactions without the addition of EDTA were not contaminated, then all three monovalent ions are indeed effective cofactors in RNA cleavage. The second reactions with EDTA showed that high concentrations of lithium, given additional time to react, have some catalytic activity. However, continued research into the mechanisms of this reaction is necessary before decisive conclusions are drawn.

MATERIALS AND METHODS

Materials

Preparation of Standardized Solutions The preparation of various standardized stock solutions was required in order to create the conditions necessary for the cleavage of RNA by DNAzymes to occur. This process required an environment of a pH of 8.5, so 25 mL of .5 Trizma buffer was prepared. Having prepared this buffer, the team also prepared various salt solutions in which the effectiveness of RNA cleavage would be tested. These included 10 mL of .1 M $MgCl_2$; 50 mL of 4 M LiCl; 50 mL of 5 M NH_4Cl; and 50 mL of 4 M KCl. Additionally, a .5 M stock solution of disodium ethylenediaminetetraacetate (Na_2EDTA) was prepared to use as a purifying agent in later tests, because EDTA chelates to divalent ions. Looking ahead toward

the process of gel electrophoresis, the team also prepared 2 L of 10X TBE, comprised of 220 g Trizma base, 110 g boric acid, and 80 mL of .5 M Na_2EDTA, in addition to 10 mL of 80% stop solution and 350 mL of cresyl violet dye solution.

Before starting the experiment, the concentration of the DNA enzyme, RNA substrate and RNA control samples had to be measured. An arbitrary absorbance of 0.5 was chosen for the DNA and RNA to use to calculate the theoretical concentration, which was determined for the sample using Beer's L
aw. Taking this concentration prediction into account, nucleic acid samples were prepared in order to perform a spectroscopy scan. The results of the subsequent scan calculated the actual absorbance of the sample. The ε_{260} of the DNA enzyme was found to be 3.0×10^5 A M^{-1} cm^{-1}; the ε_{260} of the RNA substrate was found to be 2.1×10^5 A M^{-1} cm^{-1}; the ε_{260} of the RNA product control was found to be 1.2×10^5 A M^{-1} cm^{-1}Finally, using this experimental data and Beer's Law, the exact concentrations, in comparison to the projected concentrations, were determined to be 41.7 µM for the DNA enzyme solution and 165 µM for the RNA substrate and RNA product solutions. The information obtained through this process was essential in later calculating the needed concentrations of other solutions that were to be used during the gel electrophoresis.

Reactions   Of the 10 wells available in each gel, it was decided to run six controls in each. These controls aided in the interpretation of the results of gel electrophoresis. One well contained loading buffer only, which aided interpretation by making the gel asymmetrical, enabling proper identification of the wells. A second well contained the 10-23 DNA enzyme, which, as a larger molecule, did not travel far through the gel, and left a characteristic band at the top of each well in which it was present. A third well contained RNA substrate that would be cleaved by the 10-23 DNA enzyme. Additionally, since RNA traveled faster through the gel than the DNA enzyme, the RNA also left a characteristic band underneath that of the DNA enzyme. A fourth well contained the product of RNA cleavage; as the smallest sequence, it traveled fastest through the gels, and, if the DNA enzyme did succeed in cleaving RNA, the RNA product left a characteristic band at the bottom of the gel. A fifth well contained both the 10-23 DNA enzyme and the RNA substrate to react with no cations present. The fifth well acts as a negative control since there should be no cleavage of the RNA if the cation cofactor is absent. A sixth well contained the DNA enzyme, the RNA substrate, and $MgCl_2$ solution, in order to serve as a positive control, because the 10-23 DNA enzyme cleaves the RNA in the presence of magnesium ions.

The four remaining wells in each gel were used to test the effectiveness of varying concentrations of cation cofactors. Both KCl and LiCl were present in concentrations of 1 M, 2 M, 3 M, or 3.4 M, while NH_4Cl was present in concentrations of 1 M, 2 M, 3 M, or 4 M.

Upon completion of preparation of the previous solutions, including the buffer, stop solution, and stock solutions of the salts, the actual mixtures needed in the electrophoresis (PAGE) portion of the experiment were prepared. All wells contained 20 mM Trizma Buffer. The final concentration of the 10-23 DNA enzyme described above was 41.7 µM D105 solution. The concentration of the RNA substrate was 165 µM R004 solution. The RNA product was 11 nt RNA standard. The concentration of the $MgCl_2$ in the positive control reaction was 10 mM.

The variable salts described above were prepared from diluting the 4 M KCl and LiCl stock solutions and the 5 M NH$_4$Cl stock solutions to the concentrations described above.

Oligonucleotide Preparation   Upon preparation of the solutions, they were centrifuged for approximately three seconds. The contents were then heated for two minutes in order to denature the enzymes present in the solutions. The solutions were then promptly centrifuged once again for another three seconds and then placed in an ice bucket for a total of five minutes. Upon removal from the ice, the solutions were spun down before they were incubated for thirty minutes at 37°C.

As the mixtures were being incubated, 8 µL of stop solution were measured out a total of thirty times and were each placed in a thirty microcentrifuge tubes respectively. After the thirty reacting mixtures had finished incubating for thirty minutes, 4 µL of each of the thirty solutions were measured out and added into each of the thirty microcentrifuge tubes containing the 8 µL of stop solution. The solutions were heated for two minutes before being centrifuged once again for approximately three seconds.

Table 1: Well Set-Up for Gel Electrophoresis

Well Number	Contents
1	Dye Only
2	1 M Salt (LiCl, KCl, or NH4Cl) Solution
3	2 M Salt (LiCl, KCl, or NH4Cl) Solution
4	3 M Salt (LiCl, KCl, or NH4Cl) Solution
5	4 M Salt (LiCl, KCl, or NH4Cl) Solution
6	.1 M MgCl2 Salt Solution
7	RNA and DNAzyme with No Salt Added
8	RNA Substrate Only
9	Cleaved RNA Product Only
10	DNAzyme Only

<u>Gel Electrophoresis with PAGE</u>

A ten-well 15% acrylamide urea gel from BioRad was run for each of the three experimental salt solutions tested (LiCl, KCl, or NH_4Cl), as depicted in Table 1. The wells were loaded in the order described in Table 1.   Upon completion of the loading of the gel plates, the PAGE was run at 180 volts for 35 minutes. After this was completed, the gels were soaked in cresyl violet staining dye and were de-stained in de-ionized water for 48-72 hours.

<u>Following Trials</u>

After results were obtained from this first set of gels, a second set of gels were run using the same solutions prepared previously. Additionally, two more sets of gels were run in which each well (except that containing $MgCl_2$) contained additional EDTA solution.   EDTA, which chemically bonds to the Mg^{2+} cation, was used in order to ensure that the activity of the DNAzyme was not falsely enhanced by the presence of Mg^{2+}. Having run all the tests, the PAGE gels were analyzed using a fiber-optic scanner.

RESULTS
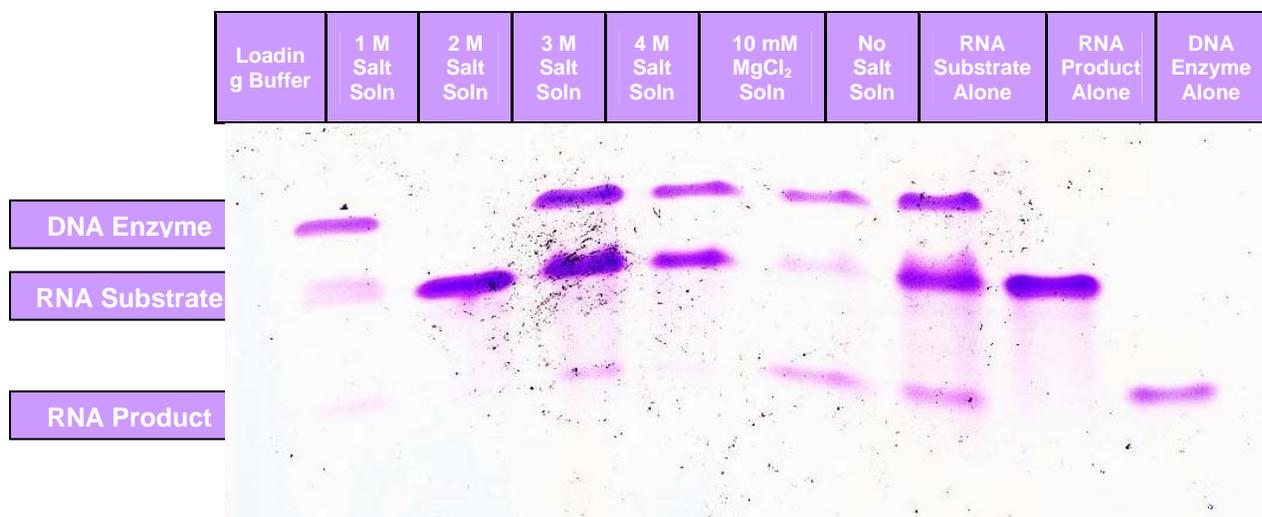
Figure 7: Image of PAGE gel containing KCl solutions without addition of EDTA. While the 3 M solution seems to be the most effective concentration of K^+ tested, it yields weak results compared to $MgCl_2$.

Figure 7 depicts the result of the 10-23 DNA enzyme's effectiveness in the presence of KCl. The first column contained loading buffer only and shows no bands because there was no DNA enzyme or RNA substrate involved. The second column containing 1M KCl has minimal cleavage with a very slight band of RNA product. The well containing 2M KCl has no band for the DNA enzyme because it was accidentally not added, and therefore no RNA cleavage. The 3M KCl shows some cleavage with a faded band of RNA product. The well containing 3.4 M KCl shows a minimal faded band of RNA product; however the band is so light that it may be regarded as having no cleavage. The $MgCl_2$ shows significant cleavage with an obvious band of

RNA product. The well containing no salt shows a good deal of RNA cleavage with a well defined band of RNA product. This control suggests that contaminating divalent cations are being introduced to the reaction mix present, mostly likely from the DNA enzyme or the RNA substrate solutions which are added. Wells containing the RNA substrate and RNA product by themselves displayed the expected banding patterns. The well that should have had only the DNA enzyme showed no band, which indicates the DNA enzyme was most likely not added to the reaction mix. Overall, even though the 3 M solution seems to be the most effective concentration of K^+ tested, it yields weak results compared to $MgCl_2$.
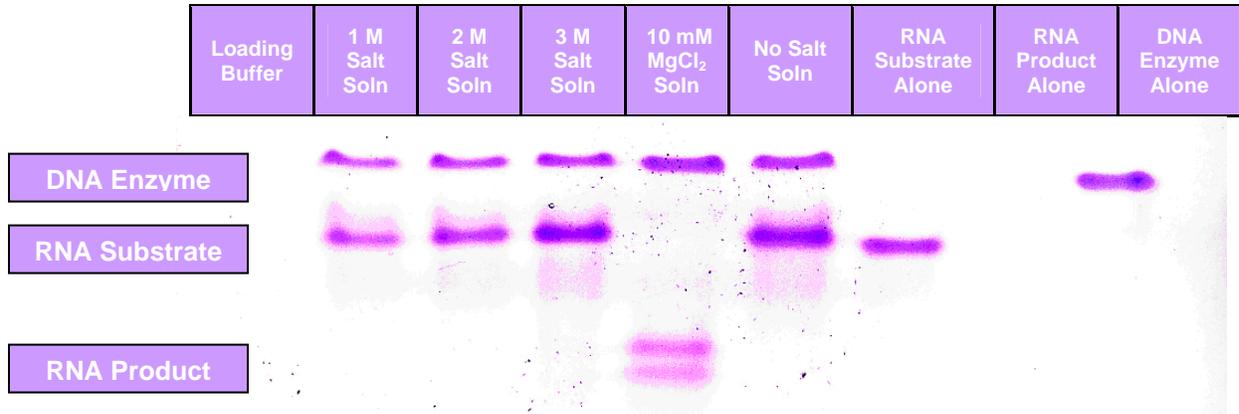
Figure 8: Image of PAGE gel containing KCl solutions with addition of EDTA; the reactions were run for 30 minutes. Given the added purifier, the lack of cleavage induced by KCl solutions indicates that this cation is not an effective catalytic cofactor in RNA cleavage.
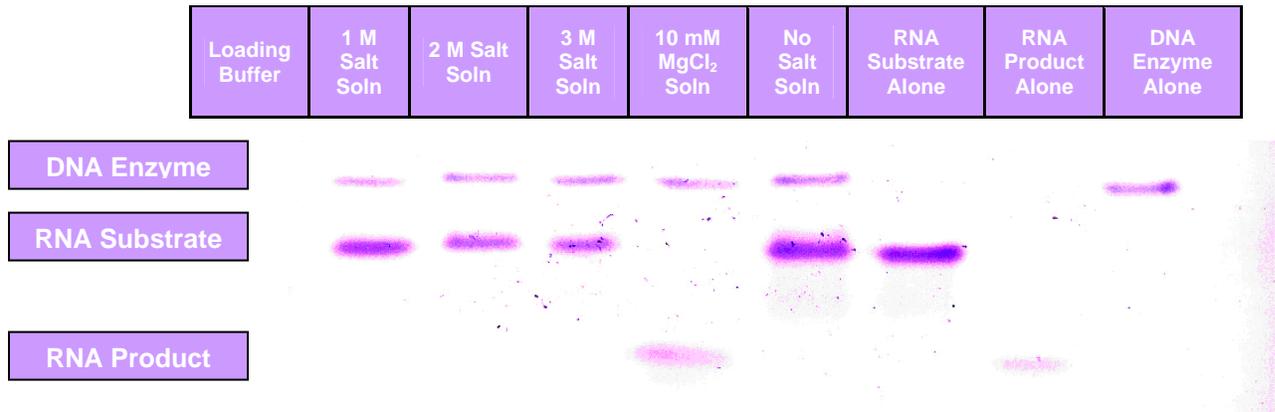
Figure 9: Image of PAGE gel containing KCl solutions with addition of EDTA; the reactions were run for 20 hours. Given the added purifier and the additional time given for the reaction, the lack of cleavage induced by KCl solutions indicates that this cation is not an effective catalytic cofactor in RNA cleavage.

[1-11]

Since it appears that divalent cations are being introduced to the reaction mix, EDTA was used to eliminate this source of contamination. Figures 8 and 9 depict the result of the 10-23 DNA enzyme's effectiveness in the presence of KCl with EDTA. While the reactions in Figure 8 had a duration of only 30 minutes, the reactions in Figure 9 were allowed to run for 20 hours. In both gels, the results reveal that the addition of 1.0 M to 3.0 M solutions of KCl to the mixture of DNA enzyme and RNA substrate did not produce any RNA product. In fact, KCl gel electrophoresis data resembles that of gel electrophoresis data from the no salt negative control. In contrast, magnesium chloride in the reaction helped generate cleavage; the final RNA product split up by the 10-23 DNA enzyme actually formed. Given the added chelating agent and the lengthened reaction time, the lack of cleavage induced by KCl solutions indicates that this cation is not an effective catalytic cofactor in RNA cleavage.

Figure 10: Image of PAGE gel containing LiCl solutions without addition of EDTA. These results indicate that Li^+ is as effective a catalytic cofactor as Mg^{2+}.

Figure 10 depicts the result of the 10-23 DNA enzyme's effectiveness in the presence of LiCl. In lane 7, there was again RNA cleavage when no additional salts were added, again suggesting divalent ion contamination of the nucleic acid samples used. In lane 1, where the loading buffer was added, there was no staining. In lane 2, the 1 M LiCl showed the most successful cleavage of the RNA substrate, gradually decreasing in effectiveness as concentration increased in lanes 3, 4, and 5. In lane 6, the $MgCl_2$ lane, there was no RNA substrate added, so no cleavage occurred. Lane 8 had the RNA substrate travel partly down the lane. In lane 9, the RNA product was not added. In lane 10, the DNA enzyme remained in its characteristic band.

	Loading Buffer	1 M Salt Soln	2 M Salt Soln	3 M Salt Soln	No Salt Soln	10 mM MgCl$_2$ Soln	RNA Substrate Alone	RNA Product Alone	DNA Enzyme Alone

DNA Enzyme

RNA Substrate

RNA Product
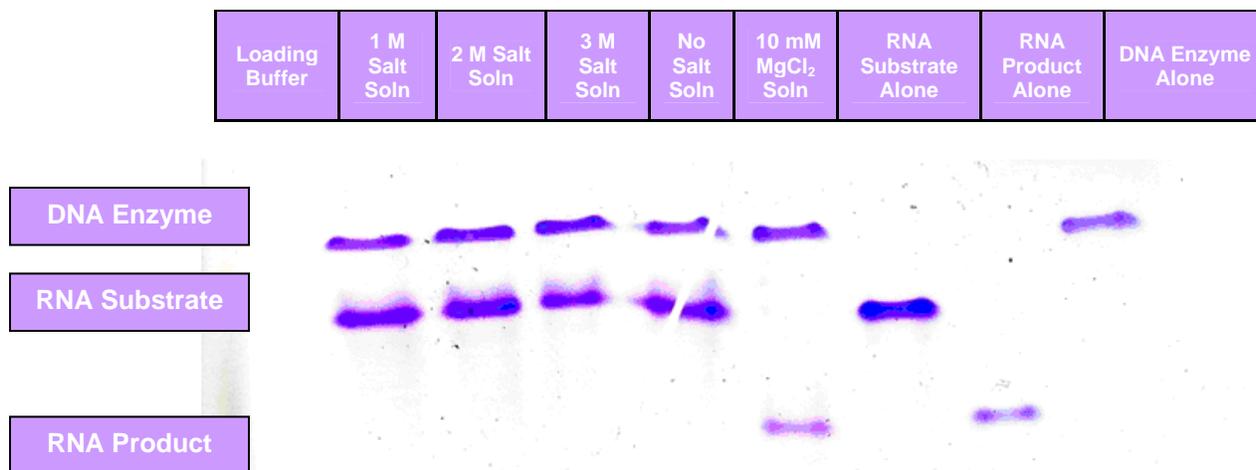
Figure 11: Image of PAGE gel containing LiCl solutions with addition of EDTA; the reactions were run for 30 minutes. Given the added chelating agent, the lack of cleavage induced by LiCl solutions indicates that this cation is not an effective catalytic cofactor in RNA cleavage.

Figure 11 depicts the result of the 10-23 DNA enzyme's catalytic mechanism in the presence of LiCl, in addition to EDTA, after the reactions were allowed to run for 30 minutes. The gel electrophoresis results reveal that addition of 1.0 M to 3.0 M solutions of LiCl to the DNA substrate/ RNA reactant mixture did not produce any RNA product. In fact, LiCl gel electrophoresis data resembles that of gel electrophoresis data from the no salt negative control. In contrast, magnesium chloride in the DNA/RNA mixture helped generate cleavage; the final RNA product split up by the 10-23 DNA enzyme actually formed. Given the added chelating agent, the lack of cleavage induced by LiCl solutions indicates that this cation is not an effective catalytic cofactor in RNA cleavage.

	Loading Buffer	1 M Salt Soln	2 M Salt Soln	3 M Salt Soln	10 mM MgCl$_2$ Soln	No Salt Solution	RNA Substrate Alone	RNA Product Alone	DNA Enzyme Alone

DNA Enzyme

RNA Substrate

RNA Product
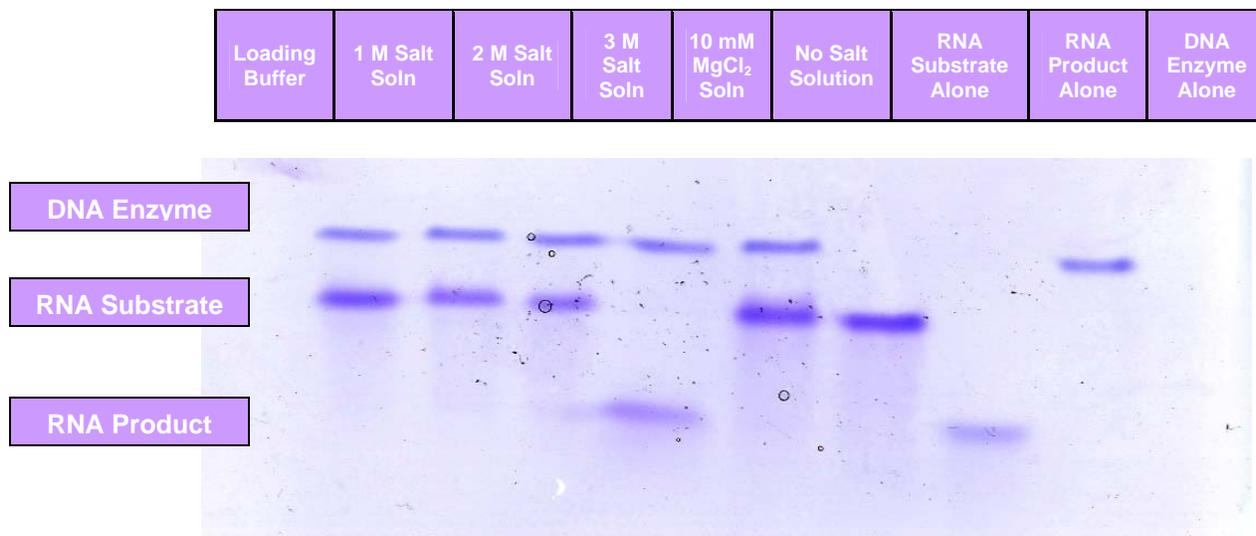
Figure 12: Image of PAGE gel containing LiCl solutions with addition of EDTA; the reactions were run for 20 hours. The slight cleavage in the 4 M solution indicate lithium ions can indeed aid in the cleaving of RNA.

Figure 12 depicts the result of the 10-23 DNA enzyme's effectiveness in the presence of LiCl with the addition of EDTA when the reactions are run for 20 hours. While there appears to be no cleavage in the 1 M, 2 M, or 3 M wells, there appears to be a faint band of RNA product in the 4 M well. However, it is difficult to distinguish whether this band is the result of the spreading of the $MgCl_2$ band next to it. If the band is indeed RNA product cleaved with the aid of Li^+ as a catalytic cofactor, these results seem to indicate that higher concentrations of monovalent salts do indeed induce greater cleavage of RNA when given a longer time to react. Regardless, Li^+ is still far less effective than Mg^{2+}; given the results of the other tests, it is apparent that the presence of $MgCl_2$ leads to the best cleavage overall.

Loading Buffer	1 M Salt Soln	2 M Salt Soln	3 M Salt Soln	4 M Salt Soln	10 mM $MgCl_2$ Soln	No Salt Solution	RNA Substrate Alone	RNA Product Alone	DNA Enzyme Alone

DNA Enzyme

RNA Substrate

RNA Product
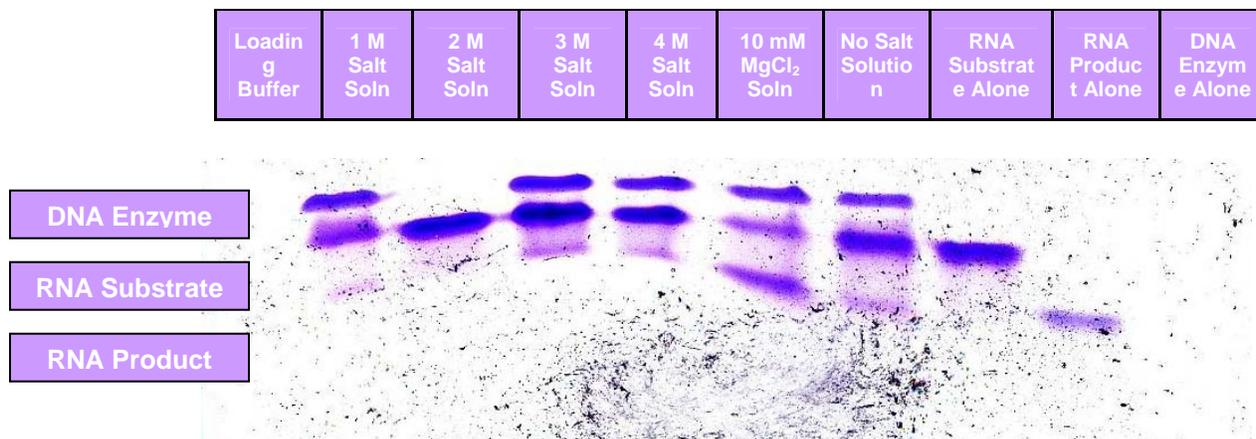
Figure 13: Image of PAGE gel containing NH_4Cl solutions without addition of EDTA. While the 3 M solution seems to be the most effective concentration of K^+ tested, it yields weak results compared to $MgCl_2$.

Figure 13 depicts the result of the 10-23 DNA enzyme's effectiveness in the presence of NH_4Cl. The well containing 1 M NH_4Cl displayed minimal cleavage. Since there is no band for the DNA enzyme in the well containing 2 M NH_4Cl, it is likely that the DNA enzyme was not in fact added, leading to no RNA cleavage. The well containing 3 M NH_4Cl led to the most effective cleavage of the four different concentrations tested. The 4 M NH_4Cl showed slightly less effective cleavage than the 3 M NH_4Cl. However, out of all the salts tested, the presence of $MgCl_2$ leads to the best cleavage overall. Additionally, the well with no salt displayed some cleavage. The RNA substrate and RNA product displayed their characteristic bands.

Figure 14 depicts the result of the 10-23 DNA enzyme's ineffectiveness in the presence of NH_4Cl, as well as EDTA, which prevents contamination of the samples by magnesium ions. As per the stains imprinted on the electrophoresis gels, the first well containing only dye did not appear. The RNA substrate, RNA product, and 10-23 DNA enzyme controls fell in accordance with what was expected. As all the wells contained EDTA, the absence of significant bands demonstrating lack of RNA cleavage in the ammonium wells indicates that this cation is not an effective catalytic cofactor in RNA cleavage. The Mg^{2+} salt solution demonstrated a high degree of cleavage, whereas the well lacking ammonium salt solution remained uncleaved.
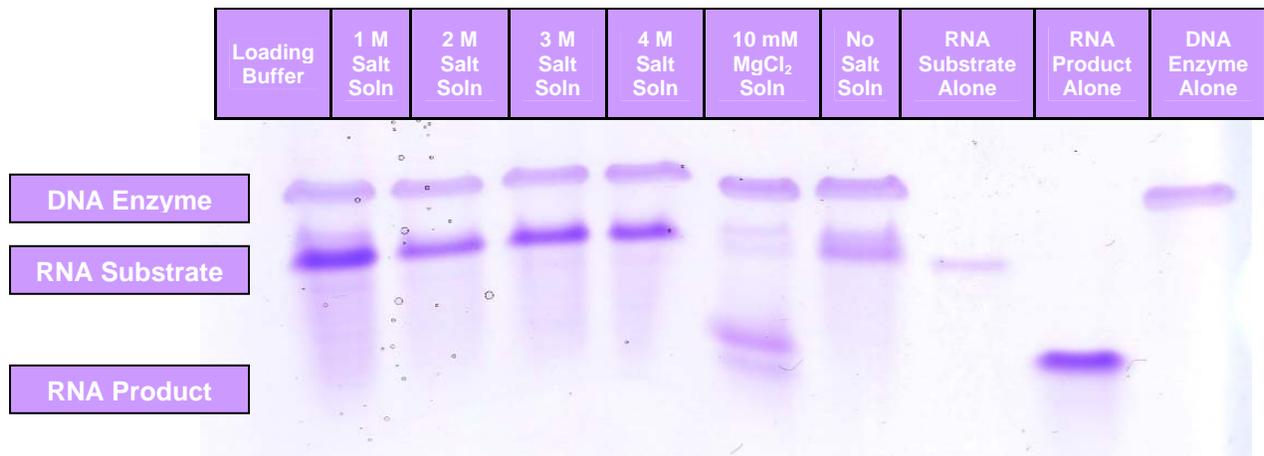
Figure 14: Image of PAGE gel containing NH₄Cl solutions with addition of EDTA. Given the added purifier, the lack of cleavage induced by KCl solutions indicates that this cation is not an effective catalytic cofactor in RNA cleavage.

DISCUSSION

While monovalent salts did not interact with the DNA enzyme with a thirty minute incubation period, analysis of lithium chloride solutions incubated for twenty-four hours may indicate RNA cleavage. Potassium chloride solutions incubated overnight did not cause any RNA intermediates or RNA product. This data implies that the rate of reaction of the DNA enzyme with monovalent salts is slower than the DNA enzyme with divalent salts like magnesium chloride. Since twenty hours was necessary to initiate even the faintest appearance of the RNA product, increased trials varying the times of reaction are necessary to successfully analyze how the 10-23 DNA enzyme works with monovalent salts.

Further observations indicate that potential cleavage of RNA only occurs if at there is at least a 3 M concentration of the monovalent cation catalytic cofactor. As a result, greater understanding of the catalytic mechanism of the DNA enzyme could be achieved with tests in which the cation concentration were greater than 4 M.

Greater analysis of the results indicates that in terms of activity, magnesium is much more effective than lithium. In the experiment, the six controls were run in order to aid in analysis of the results and hint at possible sources of contamination. One well in each gel contained the 10-23 DNA enzyme and the RNA substrate without the addition of a salt solution, in order to serve as a negative control because nucleic acid catalysts should not cleave RNA without a cation cofactor. However, in all the gels tested without the addition of EDTA, the well lacking a cation displayed cleavage. These consistent results seem to indicate that Mg^{2+} or other divalent ions were present in the DNA enzyme and RNA substrate solutions.

Essentially, our experiment affirmed the effectiveness of Mg^{2+} ions as a catalytic cofactor of the 10-23 DNA enzyme's cleavage of RNA. This is fortunate because Mg^{2+} is already present in the body in the low concentrations the mechanism requires, whereas the monovalent ions

tested were relatively less effective in high concentrations that already would be fatal to a living organism.

CONCLUSION

Ultimately data revealed that high concentrations of monovalent ions interact with the 10-23 DNA enzymes at much slower rates than divalent cations, if at all. If anything, studies proved that divalent cations are still vastly more efficient in stabilizing the DNA enzyme-RNA substrate cleavage reaction.

Comparing the mechanisms of ribozymes and DNA enzymes reveal that the RNA does not need divalent ions as much as the 10-23 enzyme does. In ribozyme catalytic activity, the hydroxyl group on the two prime position of the ribose sugar can form stable hydrogen bonds with bases and phosphates on substrate nucleotides to stabilize the enzyme's structure. In contrast, DNA enzymes have hydrogen on the two prime position of the deoxyribose sugar instead of a hydroxyl group; as a result, DNA enzymes cannot use such strong intermolecular forces to stabilize the catalytic conformation. We hypothesize that divalent ions therefore play a larger role stabilizing the DNA catalytic conformation, and therefore their function cannot be replaced by monovalent ions.

In order to further prove this claim, scientists should test for enzymatic activity in different monovalent salt concentrations beyond the alkali metals and one polyatomic cation. Further understanding of the effects of ionic radii or metallic character on 10-23 DNA enzyme activity can be achieved by greater experimentation on a wider range of salts. Lastly, in order for the 10-23 DNA enzyme to be applicable in the medicinal purposes previously mentioned, further experiments must be carried out under physiological conditions, in order to determine the feasibility of high salt concentrations in the body. Overall, greater understanding of 10-23 DNA enzymatic kinetics is necessary to apply the enzyme in the real world.

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