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

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ABSTRACT

DNA and RNA enzymes tend to act more efficiently in the presence of metal cofactor ions, such as Mg^{2+}. Whether other ions with similar chemical and structural properties catalyze DNA and RNA as successfully as magnesium ions has come into question. Using gel electrophoresis, the activity of the 10-23 DNAzyme was tested with strontium ions and cobalt hexamine. Strontium effectively enhanced the activity of the DNAzyme while cobalt hexamine does not appear to be as effective. This suggests that ligand exchange is a more important cofactor property compared to size since strontium has a larger ionic radius than magnesium while cobalt hexamine lacks the ability to exchange ligands, whereas magnesium does.

INTRODUCTION

Enzymes are organic catalysts that lower the activation energy needed for a reaction to occur. With the proper enzyme, a reaction that would normally take thousands of years to take place could be completed within several minutes. Most enzymes are proteins made up of amino acid polypeptide chains that are coiled into complex three-dimensional structures. Every enzyme reacts with a specific substrate, unique to that enzyme. This substrate corresponds to the shape of the active site, which is determined by the 3-D structure of the enzyme. The area at which an enzyme bonds to its substrate is known as the active site. After the enzyme-substrate complex is formed, the reaction is able to proceed with less activation energy. The effectiveness of an enzyme is greatly affected by the pH and temperature of its environment, as well as the presence of required cofactors; without optimal conditions, an enzyme can denature, which means it loses its shape and ability to function because it can no longer fit with its substrate (1). Enzymes, like all catalysts, are not used up in a reaction; they merely create an intermediary state in the reaction that requires less activation energy and allows the reaction to move forward at a faster rate.

It was previously assumed that only proteins had the capacity to catalyze reactions, due to their complex folded structures (2). However, in recent years, it was discovered that RNA can act as an effective catalyst in some reactions; these RNA molecules are called ribozymes (9). However, catalytic activity was not limited to proteins and RNA but was also found to occur in specialized DNA called DNAzymes. It was found that DNAzymes, though not found in nature, could be created *in vitro* (2). These ribozymes and DNAzymes bind to their substrates via site specific base pairing. Each ribozyme and DNAzyme has a specific base pair sequence that corresponds with the structure of that enzyme (3). When in the presence of these specific DNA or RNA sequences, the enzymes can bind to nucleic acid sequences and perform actions such as

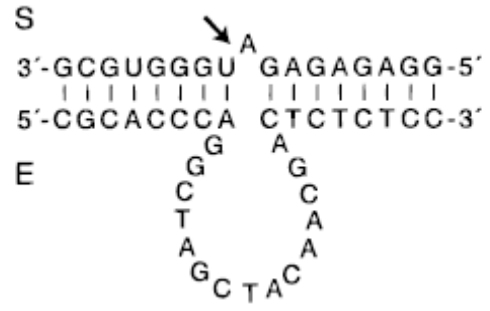

Figure 1: Secondary structure of the site at which site-specific 10-23 enzyme attaches and cleaves the RNA (1).

cleavage of that nucleic acid strand. One specific DNAzyme that was created was the 10-23 DNAzyme. These DNAzymes were synthesized in the laboratory with a specific nucleotide sequence in order to cleave RNA (3). At the point of attachment, the DNAzyme forms a loop that extends from the RNA strand (1). This loop allows for the proper aligning of the base pairs of the RNA strand at this specific attachment sequence (Fig. 1). The RNA is then cleaved by the DNAzyme, yielding two strands of RNA, one that is 9 base pairs (bps) long and the other that is 11 bps long. The DNAzyme loop expands, separating the two split RNA sequences (1). However, these reactions are very slow, and would not proceed without cofactors.

Cofactors bind to the enzyme at its active site and modify the shape of the enzyme, allowing it to perform at its maximum potential. Both DNA and RNA include a negatively charged phosphate backbone. Thus, the phosphate group of the 10-23 DNAzyme would repel from the similarly charged group on the RNA substrate. Therefore, the DNAzyme requires a cation cofactor to mitigate the electrostatic repulsion forces that would result from close proximity between two strands of DNA and RNA. In the presence of metal ion cofactors, DNAzyme attaches onto the purine located on the shorter RNA strand and cleaves it at this site (Fig. 1) (4). Without cofactors, this process is much less effective.

The precise function of the metal cation and its mechanism are not fully understood. However, specific interactions between these metal cation cofactors of ribozymes have been investigated (5). Understanding the importance and function of these cofactors allows researchers to have a better understanding of how ribozymes work. The purpose of this experiment is to engage in one small step of applying those same strategies to DNAzymes. The ability to compare how RNA and DNA enzymes work is a very interesting question, since they are structurally similar at a monomeric level but not at the polymer level.

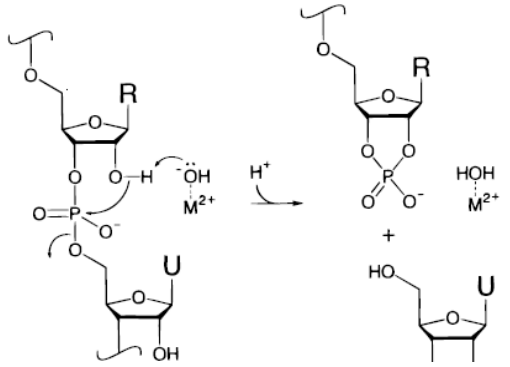

Figure 2: Ligand interaction hypothesis of the function of the metal cation (1).

One hypothesis regarding the function of the metal cation emphasizes the importance of ligand exchange. Ligands are substituent molecules weakly bonded to a central ion. These ligands can sometimes be exchanged for other substituent groups. Magnesium ions, cations that have been shown to be effective cofactors, form ligands with the hydroxyl groups of the water molecules around it (1). When this cation with ligands interacts with the nucleic acid, the hydrogen from one of the hydroxyl groups is removed from the nucleic acid. The hydrogen then

attaches to the hydroxyl group of the metal-ligand complex, forming a water molecule. The resulting RNA has an electronegative oxygen that is attracted to the phosphorous in the phosphate group. This oxygen replaces the oxygen-phosphorous bond that previously connected the two nucleotides, breaking the phosphodiester linkage (Fig. 2) (1).

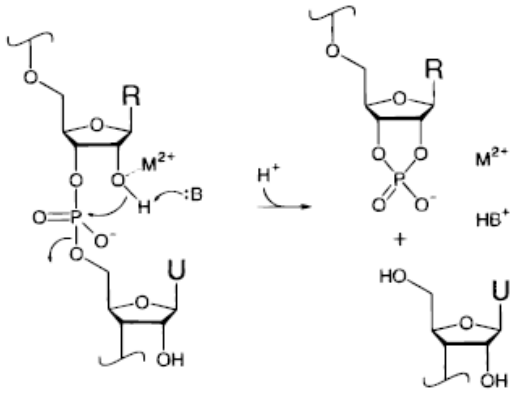

Figure 3: Ligand interaction hypothesis of the function of the metal cation (1).

The other hypothesis is that the mechanism of the reaction does not relate to the ligands at all, but to the size of the ion and where it can attach on the nucleic acid. Magnesium is a relatively small ion. A molecule that is too large would not be able to attach to the nucleic acid. Therefore, it is just the right size to replace the hydrogen on the hydroxyl group. The hydrogen from the hydroxyl group of the nucleic acid then interacts with other ions in the solution, and is removed from the nucleic acid. A bond forms between the unbounded electrons on the oxygen and the phosphate group, breaking the phosphodiester bond (Fig. 3) (1).

The study of these nucleic acid enzymes is significant because a better understanding of these compounds can lead to many other important discoveries and inventions. For example, the site-specific cleavage ability of the DNAzyme has potential medicinal applications. DNA and RNA enzymes can be used to target and cleave specific strands of RNA that have negative effects, such as viral RNA (6). Additionally, a better understanding of nucleic acids and proteins can help scientists understand the development of organisms on a biochemical level. Specifically, a study of the 10-23 DNAzyme might produce a better understanding of the puzzling "chicken-and-the-egg" scenario regarding DNA, RNA, and enzymes; namely, which came first, second, and third in the development of life (3). As DNA is extremely efficient at storing information, and proteins are effective when catalyzing reactions, examining RNA as a possible intermediary form – that is, one that can do both – provides insight into the origins of life. Seeing which functional aspects of RNA are more similar to either DNA or proteins can help determine the path that early life took in its development.

The aim of this experiment is to better understand the 10-23 DNA enzyme; specifically, the goal is to determine the characteristics that a cofactor requires to help catalyze this reaction. Previous experiments have concluded that the Mg^{2+} ion has successfully catalyzed this reaction, but it is unknown what specific characteristics make this ion such a good cofactor. In these previous experiments, monovalent salts were repeatedly unsuccessful and ineffective in catalyzing the reaction whereas divalent salts such as magnesium showed positive results (7). The objective of this project is to determine what properties of magnesium make it such a good cofactor. It is unknown whether the size of the ion or its ability to exchange ligands is the main contributing factor. To determine which is more important, the experiment tested these two variables: size and ligand exchange. In an attempt to better understand the size aspect, strontium ions were tested. Strontium ions have larger ionic radii than the magnesium ions, so the success of the strontium in the reaction would help support the hypothesis that the ion's size is not the most important factor for the reaction's success. In addition, the effects of cobalt hexamine, an

ion that does not have the ability to exchange ligands, were tested. Any positive results of the cobalt hexamine reactions would prove that ligand exchange is not necessary for the 10-23 DNAzyme to function.

Gel electrophoresis will determine if these ions can successfully catalyze the reaction. The first few lanes contain controls that act as markers in order to determine what the bands mean. These lanes contain the DNAzyme, RNA substrate, and an ideal RNA product standard. If the reactions are successful, the other lanes on our gel will contain three bands. One band will be for the DNAzyme, the other for the RNA substrate, and the latter for the RNA product produced when the RNA substrate is cleaved by the enzyme. If the ions are not able to catalyze the reaction, then a band will be visible on the same horizontal plane as the RNA substrate control and there will be no bands on the same plane as the RNA product standard. Gel electrophoresis will help determine if the reaction is a success by determining the presence of cleaved RNA product. The visibility of the bands on the gel will also help us quantify the amount of RNA product created after the reaction. Darker bands indicate that the ions are more effective in catalyzing the reaction. In contrast, lighter bands indicate that the ions are less effective in catalysis.

In the reactions for strontium and cobalt hexamine ions, varied results are expected. Both ions are expected to be successful cofactors in catalyzing the reaction. However, it is also believed that strontium will be more successful than cobalt hexamine and will produce more RNA product because of its ability to exchange ligands. Moreover, this project will test various concentrations of the ions in the reactions and it is expected that higher concentrations of the ions will result in more RNA product.

MATERIALS AND METHODS

Preparation of Stock Solutions

A running buffer of tris-borate-EDTA (0.01M EDTA pH8, 0.44M Boric acid, 0.45M Tris) was prepared as a 5x stock solution. A 0.5M EDTA solution was also prepared in order to chelate any undesired metal cations. Loading buffer (0.1M formamide, 0.001M EDTA, and 0.003M bromophenol blue) was prepared. Stock solutions of 1M $SrCl_2$ and 0.2 M $Co(NH_3)_6$ were prepared. 500 mM EPPS buffer of pH 7.43 was prepared for use as a buffering agent in the reaction solution. In addition, stock solutions of 5M NaCl and .3M $MgCl_2$ were prepared.

Verification of Nucleic Acid Concentration

The concentrations of RNA substrate and DNAzyme stock solution were calculated through the use of the spectrophotometer and Beer's Law, $A = \varepsilon cl$, where A is the absorbance, ε is the absorpsivity constant, c is the concentration of the solution, and l is the path length (maintained at a constant 1cm). The absorpsivity constant of the DNAzyme is 241200 $Lmol^{-1}c^{-1}$, and the absorpsivity constant of the RNA susbtrate was 190500 $Lmol^{-1}c^{-1}$. The written concentration of the solution was used to create a solution that would yield an absorbance between 0.1 and 1. From this, the actual concentration of the stock solution was found to be approximately 385μM for the RNA substrate and 120μM for the DNAzyme. Using this value,

the amount of RNA substrate and DNAzyme to be used in the dilutions were calculated for a total reaction volume of 10μL, and these dilutions were prepared.
The DNAzyme sequence used was: CGCACCCAGGCTAGCTACAACGACTC, and the RNA substrate sequence used was: GGCGGAGAGAGAUGGGUG.

DNAzyme and RNA Substrate Reaction Setup

Molarities of 10mM, 50mM, 100mM, 150mM, 200mM, and 250mM were used for strontium chloride, and molarities of 10mM, 25mM, 50mM, 75mM, 100mM, 130mM, and 140mM were used for the cobalt hexamine. Each reaction contained 3.85 μM RNA substrate and .05M EPPS buffer were first added to each reaction. Next, the amount of stock metal cation solution necessary to achieve the desired concentrations of these cations was added. NaCl was added to create a 2M solution. 12 μM DNAzyme solution was added last in order to ensure that the reaction did not occur before all the reactants were added. These reactions were then placed in a water bath set at 37^{oC} for 1 hour in the first set of trials, and for 24 hours in future trials. In order to stop the reactions, 15 μL of loading buffer were added to each of the solutions, and the solutions were stored on ice.

Running and Staining Gels

Several 10-lane 15% polyacrylamide-urea gels from BioRAd with urea were used. Samples were loaded with standards of 12 μM DNAzyme, 3.85 μM RNA substrate, and 1.5 μM RNA product. These standards acted as a guide to show where each nucleic acid band would appear in the gel. In addition, a 10 mM reaction solution of $MgCl_2$, DNAzyme, and RNA substrate, was used as a positive control for each trial. A negative control of only DNAzyme and RNA substrate was also created and run for the trials. The negative control ensured that the reaction was not merely occurring on its own, without the use of cofactors. Similarly, the positive control was used to ensure that the reaction would occur with the proper cofactor, as magnesium has shown to enhance DNAzyme activity in the past. Each of the reactions samples were loaded into separate wells. Two trials of each metal cation were performed for each of the concentrations of the cations. In the first trials, the gels were run at 180 V for 45 minutes. In the second trials, the time was extended to one hour in order to allow for better separation of bands. The gels were stained for 10 minutes in SYBR-Green stain in the first trials, and for 30 minutes in the later trials to allow for greater visibility of the bands. The gels were analyzed on a Hitachi FMBio II machine that calculated the percent composition of RNA product in each band.

RESULTS

Trial One Gels

Trial one tested varying concentrations of both Sr^{2+} and $[Co(NH_3)_6]^{3+}$. The DNAzyme and RNA substrate reactions were incubated for one hour at 37^{o} C and the gels were run for 45 minutes. The gels were stained in SYBR-green for 10-20 minutes.

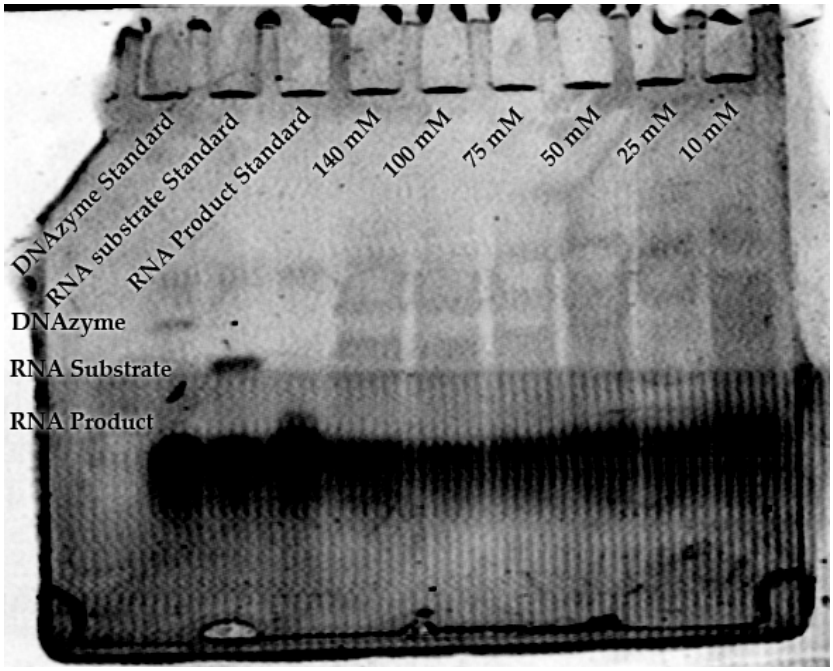

Figure 5a: Cobalt Hexamine Gel

Figure 5a depicts a gel electrophoresis for the first cobalt hexamine trial. The gel shows the splitting of RNA substrate in the presence of DNAzyme under varying concentrations of $[Co(NH_3)_6]^{3+}$. From left to right, the first three wells contained: DNAzyme, RNA substrate, and RNA product standard. Wells 4 through 9 contained concentrations of $[Co(NH_3)_6]^{3+}$ ranging from 140 to 10 mM, going from highest to lowest concentration.

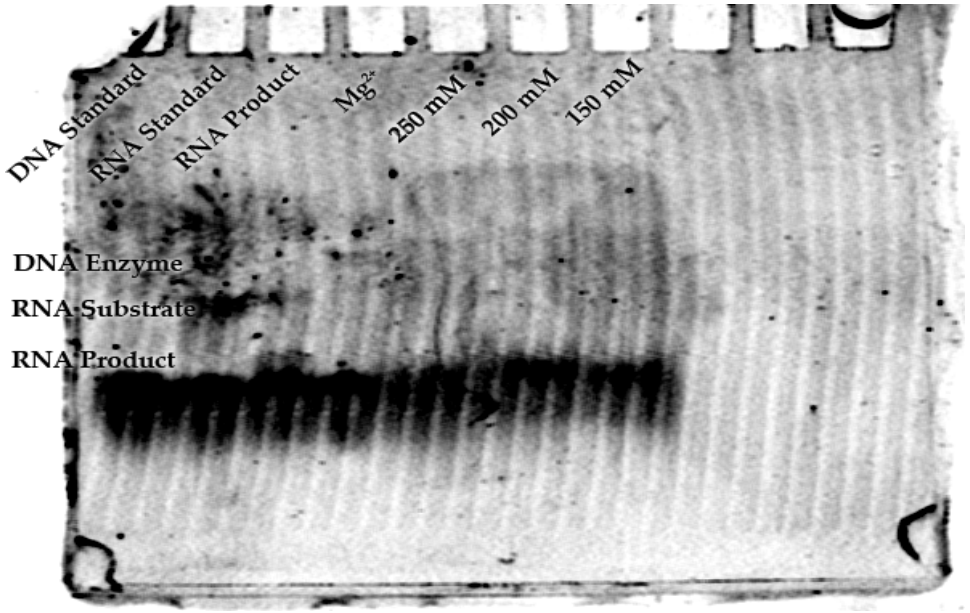

Figure 5b: Strontium Gel

Figure 5b depicts a gel electrophoresis for the first strontium trial. The gel shows the splitting of RNA substrate in the presence of DNAzyme under varying concentrations of Sr^{2+}.

From left to right, the first three wells contained: DNAzyme, RNA substrate, and RNA product standard. The fourth well contains a positive control. The concentrations of Sr^{2+} range from 250 to 150 mM, from highest to lowest concentration in wells 5-7.

The absence of the RNA product bands in lanes 4-10 of Figure 5a and in lanes 5-7 of Figure 5b leads to the conclusion that Sr^{2+} and $[Co(NH_3)_6]^{3+}$ didn't catalyze the reaction. However, the RNA product bands maybe be obscured by the large smear of bromophenol blue dye near the bottom of the gel. Overall, both gels in Figure 5a and 5b provide no evidence of RNA cleavage under trial conditions.

Trial Two Gels

In trial two, varying concentrations of Sr^{2+} and $[Co(NH_3)_6]^{3+}$ were once again tested as cofactors in the DNAzyme and RNA substrate reaction. Although reaction conditions remained consistent, the running time for the gel electrophoresis was increased from 45 minutes to one hour. Additionally, the SYBR green staining time was increased from 10-20 minutes to 30 minutes.

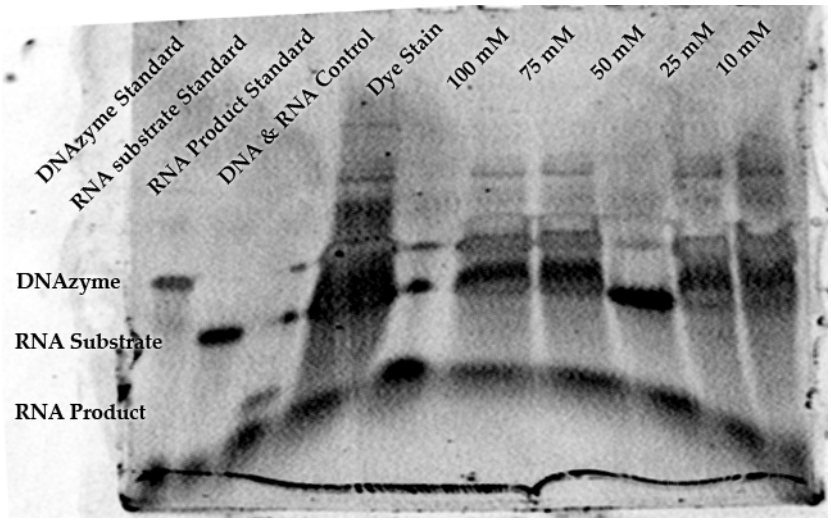

Figure 6a: Cobalt Hexamine Gel

Figure 6a depicts a gel electrophoresis for the second cobalt hexamine trial. The gel shows the splitting of RNA substrate in the presence of DNAzyme under varying concentrations of $[Co(NH_3)_6]^{3+}$. From left to right, the first three wells contained: DNAzyme, RNA substrate, and RNA product standard. The fourth well contains a negative control. Additionally, the fifth well was filled with bromophenol blue in order to determine if the previously observed smear was caused by the dye. The concentrations of $[Co(NH_3)_6]^{3+}$ range from 100 to 10 mM in lanes 6-10, from highest to lowest concentration.

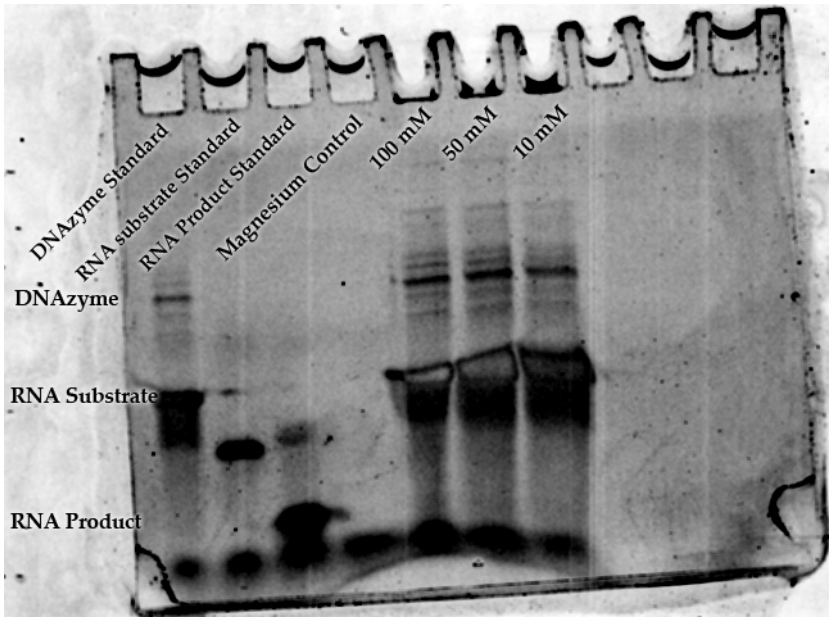

Figure 6b: Strontium Gel

Figure 6b depicts a gel electrophoresis for the second strontium trial. The gel shows the splitting of RNA substrate in the presence of DNAzyme under varying concentrations of Sr^{2+}. From left to right, the first three wells contained: DNAzyme, RNA substrate, and RNA product standard. The fourth well contains a positive control. The concentrations of Sr^{2+} range from 100 to 10 mM in lanes 5 through 7 from highest to lowest concentration.

The absence of the RNA product bands in lanes 6-10 of Figure 6a and in lanes 5-7 of Figure 6b once again shows that Sr^{2+} and $[Co(NH_3)_6]^{3+}$ did not succeed in catalyzing the reaction. Due to the longer running time of the gel, there is some separation between the RNA product standard band and bromophenol blue dye in the lane 3 in both figure 6a and 6b. The band in lane 5 in Figure 6a confirms our assumptions that bands at the bottom of the gel were leftover bromophenol blue.

Trial Three Gels

In trial three, Sr^{2+} and $[Co(NH_3)_6]^{3+}$ were once again tested at varying concentrations in the DNAzyme and RNA substrate reaction. The reaction conditions were modified from the previous two trials. The reaction incubation time was increased from one hour to 24 hours, while the temperature remained at 37^{o}C. Additionally, the gel running time was increased to approximately 1 hour and 10 minutes. This ensured that the bromphenol blue dye would run off the gel and allow for clearer RNA product bands. Finally, the SYBR-green staining time was increased to 45 minutes.

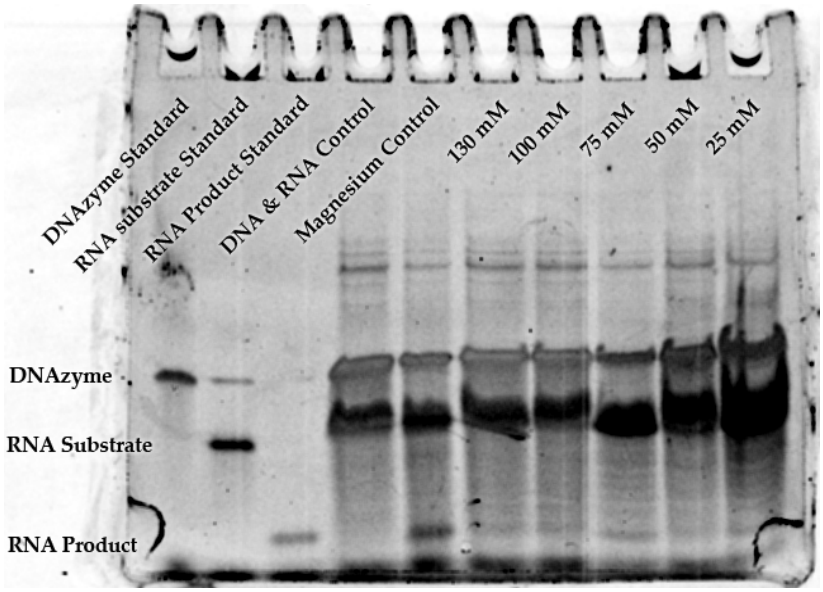

Figure 7a: Cobalt Hexamine Gel

Figure 7a depicts a gel electrophoresis for the third cobalt hexamine trial. The gel shows the splitting of RNA substrate in the presence of DNAzyme under varying concentrations of $[Co(NH_3)_6]^{3+}$. From left to right, the first three wells contained: DNAzyme, RNA substrate, and RNA product standard. The fourth well contained a negative control of DNAzyme and RNA substrate, and the fifth well contained a positive control of DNAzyme, RNA substrate, and Mg^{2+} as a cofactor. The concentrations of $[Co(NH_3)_6]^{3+}$ range from 130 to 25 mM in lanes 6 through 10, from highest to lowest concentration.

The presence of RNA product bands in lanes 6-10 of Figure 7a indicates that $[Co(NH_3)_6]^{3+}$ was successful in catalyzing the reaction between the DNAzyme and the RNA substrate. However, the product bands for these lanes are much lighter than the RNA product band for lane 5 (positive Mg^{2+} control). This indicates that there is a higher concentration of RNA product in lane 5 and that $[Co(NH_3)_6]^{3+}$ was less effective that magnesium in this trial.

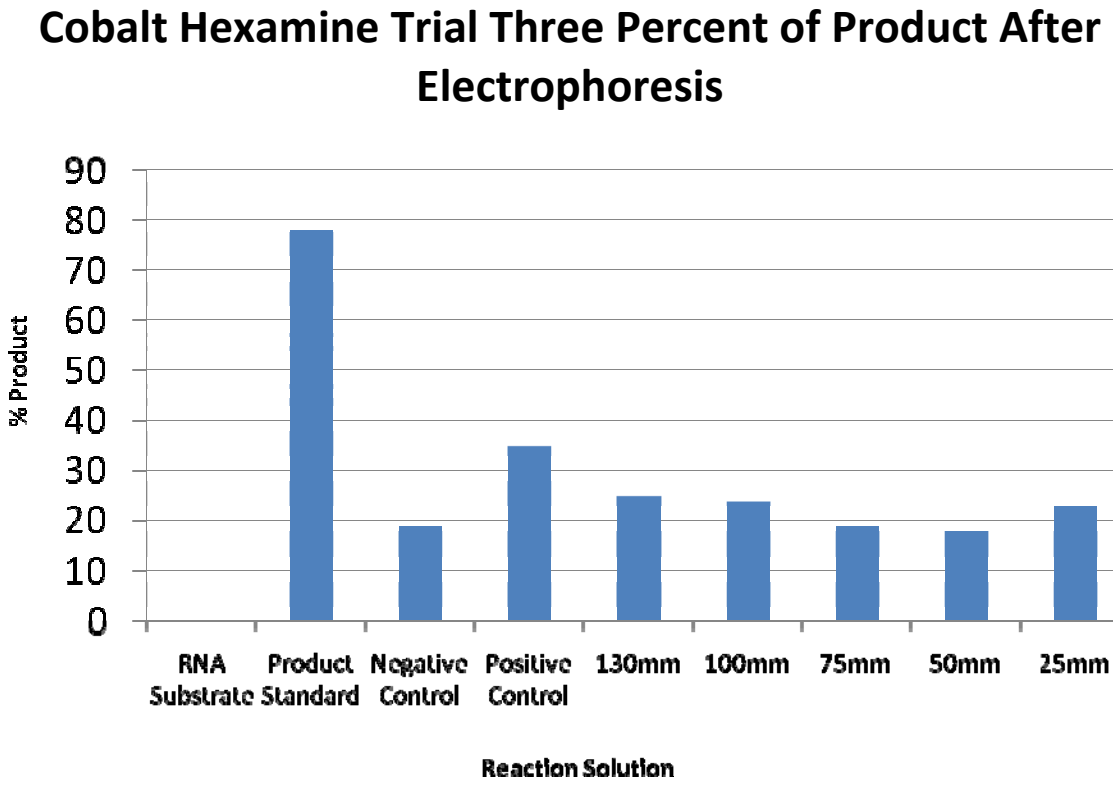

Figure 7b: Percentage RNA Product Formation after Electrophoresis of Cobalt Hexamine Gel

The percentage of RNA product formed in the cobalt hexamine gel is displayed in Figure 7b. Using Hitachi software, the volume of RNA product was measured for each band in the $[Co(NH_3)_6]^{3+}$ gel (Fig. 7a). The graph above displays the percentage of RNA product increase compared with the amount of RNA substrate present before the reaction for each reaction solution.

The positive magnesium control produced more RNA product than the other $[Co(NH_3)_6]^{3+}$ reaction solutions. The percent RNA product seems to increase with increasing concentrations of $[Co(NH_3)_6]^{3+}$. However, there is not a significant difference between percent RNA product produced by negative control and $[Co(NH_3)_6]^{3+}$, regardless of concentration. In fact, some of the lower $[Co(NH_3)_6]^{3+}$ concentrations did not surpass the negative control in percentage production of RNA product.

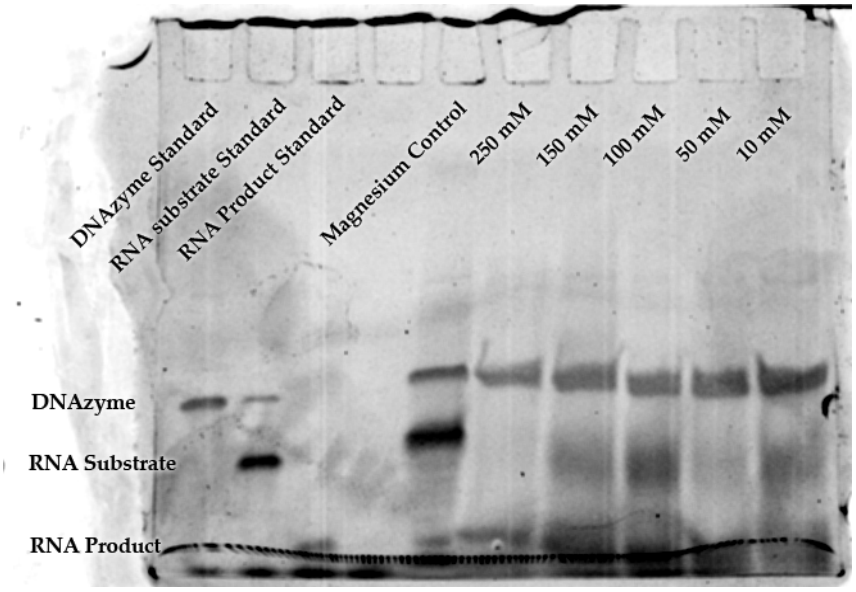

Figure 8a: Strontium Gel

Figure 8a depicts a gel electrophoresis for the third strontium trial. The gel shows the splitting of RNA substrate in the presence of DNAzyme under varying concentrations of Sr^{2+}. From left to right, the first three wells contained: DNAzyme, RNA substrate, and RNA product standard. The fifth well contains the positive control. The concentrations of Sr^{2+} range from 250 to 10 mM, from highest to lowest concentration.

The presence of RNA product bands in lanes 6-10 of Figure 8a indicates that Sr^{2+} was successful in catalyzing the reaction between the DNAzyme and the RNA substrate. The comparative darkness of the product bands indicates the concentration of RNA product formed. Additionally, in 6-10 the RNA substrate bands were not as visible showing that substrate was cleaved in the reaction.

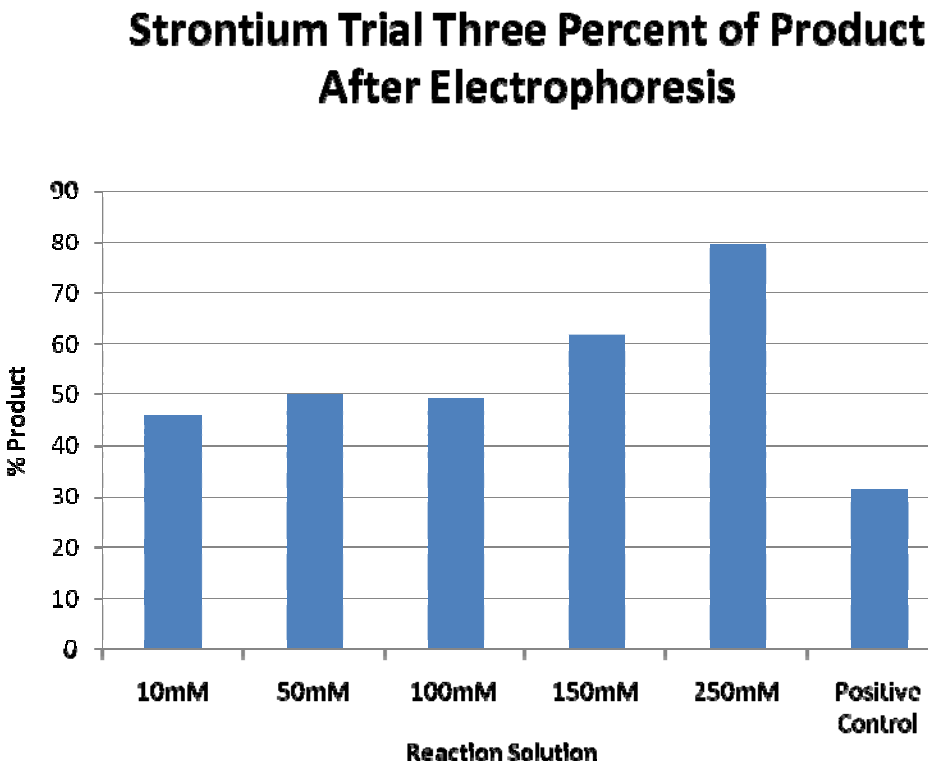

Figure 8b: Percent RNA Product after Electrophoresis of Strontium Gel

The percentage of RNA product for each lane of the strontium gel is displayed in Figure 8b. Using Hitachi software, the volume of RNA product was measured for each band in the Sr^{2+} gel (Fig. 8a). The graph above displays the percentage of RNA product increase compared with the amount of RNA substrate present before the reaction for each reaction solution.

The positive magnesium control produced less RNA product than all the Sr^{2+} reaction solutions. For example, the positive control yielded approximately 31% RNA product while the percent RNA product produced by Sr^{2+} ranged from 46 to 79% (Fig. 8b). The graph shows that percent RNA product seems to increase with increasing concentrations of Sr^{2+}.

DISCUSSION

The most vital factor to the success of the DNAzyme's catalytic ability is the time alotted for the reactions to run. In the first two trials, the reactions between the 10-23 DNAzyme and RNA substrate in the various salt environments ran for one hour (Figs. 5a-6b). In these two trials, the positive magnesium control and product standard lanes produced a product band; all the other experimental lanes for strontium and cobalt hexamine lacked product bands yet possessed full RNA substrate bands (Figs. 5a-6b). These initial results suggested that neither strontium ions nor the cobalt hexamine ions were effective cofactors of the DNAzyme. However, in trial three, extending the reaction time to 24 hours caused product bands to appear in varying degrees within most experimental lanes (Figs. 7a and 8a). For instance, the experimental strontium lanes produced solid product bands (Fig. 8a). Additionally, they lacked RNA substrate bands (Fig. 8a). This implies that strontium is an effective cofactor and can aid the DNAzyme. The quantitative

data collected from the FMBio Analysis program supports this notion because all strontium lanes contained a higher percentage of the RNA product than the positive magnesium control (Fig. 8b). In contrast, cobalt hexamine displayed thinner product bands and thicker RNA substrate bands, implying that it was not an effective cofactor (Fig. 7a). Again, this is quantified by the data from the FMBio Analysis program, in which the cobalt hexamine percent of product was not significantly different from that of the negative control (Fig. 7b). Nevertheless, the faint product bands produced by the cobalt hexamine in trial three led to the conclusion that the DNAzyme and RNA substrate reaction produces more results if allowed to react for 24 hours. Thus, the extension of the reaction time and the resulting effects suggest that the reaction requires more than one hour to fully occur and to cut up the RNA substrate.

Another important factor is the amount of time allotted for the PAGE gels to run. The position of the bromophenol blue dye helped to determine when to stop the gels because separation is needed between the RNA product and the bromophenol blue dye. In the first two trials, excess bromophenol blue dye collected near the bottom of the gels. This extraneous material created noticeable problems in trial one because it ran together with the product band. By extending the run-time of the gel from 45 minutes to one hour for trial two, more separation between the bromophenol blue band and the product band occurred. This procedural modification produced more defined and separated product bands from the excess bromophenol blue; however, in some lanes, the product bands still remained attached to the superfluous materials. Thus, in trial three, the gels ran until bromophenol blue dye began to run off the gels; this added approximately 10 minutes to the previous running time. This alteration clearly impacted the gels' clarity; all product bands were visibly distinct and separated from the remnants of the bromophenol blue dye that failed to run off the gel. Therefore, increasing the running time of the gels improved the clarity of the results, specifically, that of the product bands.

In addition to gel run time, the time allocated for the gels to stain in the SYBR green dye was directly related to the clarity of the gels. In trial one, there was less particle disturbance and more distinct bands in the gels stained in SYBR green for 20 minutes compared to those stained only 10 minutes. Thus, for trial two, the SYBR green staining time was extended to 30 minutes in order to reduce particle disturbance and improve band clarity. These procedural changes produced clearer gels; however, the gels still exhibited perceivable interference upon scanning. Therefore, the staining time was increased yet again to 40 minutes for trial three. This modification supports the notion that increased staining time led to clearer gels, as evidenced by the palpably more interpretable results. Staining the gels for a longer time darkens the bands thereby increasing the contrast between the bands and the gel surface. The increased contrast reduces the amount of magnification needed in turn preventing the augmentation of the noise on the gel surface. Thus, trial-by-trial augmentation of the SYBR green staining time resulted in more distinctive gels.

In conclusion, strontium, which can exchange ligands but is larger than magnesium, was a more viable cofactor for RNA cleavage. Therefore, it can be inferred that ligand exchange is the more important property than size. This is because the product bands from the cobalt hexamine solutions were much lighter than those produced from the strontium salt solutions. In addition, the RNA substrate bands in the strontium gels appeared much more faded than those of

the cobalt hexamine gels, which were in the same horizontal plane as the RNA substrate control band. However, the slightly visible product bands from the cobalt hexamine gels suggest that ligand exchange affects the efficacy of the site-specific cutting of RNA substrate yet is not a necessity for the functionality of the DNAzyme. Size does not appear to affect the function of the DNAzyme; however, this conclusion in part belies the results from previous research on divalent metal ion cofactors for the 10-23 DNAzyme (1). With these results, it can be concluded that ligand exchange is more important than size in metal ion cofactors.

Further experimentation can be done to more specifically determine the effects of varying divalent salt concentrations and ion size. The experimental lanes did not consist of a vast variation of salt concentrations; thus, conclusions on the optimal salt concentrations cannot be determined. Consequently, future tests can target a wider range of salt concentrations and attempt to determine the ideal concentration ranges for each of the metal ions. Additionally, larger divalent ions, such as Ba^{+2}, can also be tested to gather further information regarding the importance of the size of cofactors in DNAzyme functioning.

No previous research is known to have been conducted on the effectiveness of cobalt hexamine as a cofactor for the 10-23 DNAzyme; however, it was proven that cobalt hexamine is an effective cofactor for the hairpin ribozyme which cuts RNA in a similar fashion as the 10-23 DNAzyme (8). Previous research conducted on the effectiveness of the divalent strontium ion as a 10-23 DNAzyme cofactor showed weaker product bands. To be certain about the effectiveness of the strontium cofactor, more trials should be performed.

Additionally, these findings on the cofactor requirements for the 10-23 DNAzyme could be tested on other RNA-cutting enzymes, such as the 8-17 DNAzyme. Variables from this experiment, such as the necessity of ligand exchange, can be tested with the 8-17 DNAzyme to determine whether different forms of DNAzymes all require cofactors with similar properties or whether they require metal ions with different characteristics. Previous research conducted on the 8-17 DNAzyme has shown that magnesium ions work as an effective cofactor. Since strontium mimicked the effectiveness of magnesium ions as a cofactor the 10-23 enzyme, future testing can be conducted to determine whether strontium or ions with strontium's properties can also be effective cofactors for the 8-17 DNAzyme (10). Therefore, further experimentation can be done in order to determine the extent to which this research applies to other RNA-cutting enzymes. This future research would better define the necessary properties of cofactors for various DNAzymes and would augment the scientific community's knowledge on the enzymatic properties of nucleic acids.

CONCLUSION

The purpose of the experiment was to better understand the 10-23 DNAzyme and to determine the correct conditions and the ideal ion concentrations required for the DNAzyme to perform its specified function of cutting up RNA. The 10-23 DNAzyme has been proven to be catalyzed in the presence of Mg^{2+} ion, but it is unknown whether the size of the ion or its ability to perform ligand exchange is the main contributing factor to its success in catalyzing the reaction. Therefore, this experiment was designed on the basis of these two variables. In order to determine which variable was more important for the reaction to occur, the effects of strontium

and cobalt hexamine ions were tested. The strontium ion has a larger ionic radius than the magnesium ion, so the success of strontium ion in the reaction would proves that the size of the magnesium is not the most important factor that leads to the success of the reaction. On the other hand, cobalt hexamine does not have the ability to perform ligand exchange so any results would prove that ligand exchange is not necessary for the 10-23 DNAzyme to work. Also, we wanted to determine the specific conditions that would make the DNAzyme reach its full potential. We realized that increased reaction time made the DNAzyme more successful in cutting the RNA producing positive results.

For each ion, different concentrations were tested in the reactions to determine which concentrations would be more effective. In the strontium trials, it was determined that higher concentrations of strontium resulted in more substrate being converted to RNA product (Fig. 15). At the same time, all trials of strontium produced positive results far surpassing those of the magnesium control. With these results, it can be concluded that ion size does affect the ability of the DNAzyme to cut RNA. Because strontium has a much larger ionic radius than magnesium and had produced far more product than the magnesium control, it can be determined that the larger ionic size has a positive effect on the final results of the reaction.

In the cobalt hexamine trials, the ion catalyzed the reaction but the amount of product created was fairly small (Fig. 16). In comparison to the positive control, cobalt hexamine was not as effective in catalyzing the reaction. Only under extremely high concentrations, ones that bordered on the edge of cobalt hexamine's solubility, was the ion able to contribute to RNA catalysis. For most of the trials, if cobalt hexamine was not above a certain concentration, its ability to catalyze the reaction was similar to the negative control. Therefore, cobalt hexamine is not very effective as a catalyst unless it is present in high concentrations. Cobalt hexamine's disappointing results in comparison to the positive control or strontium ion can be attributed to its inability to undergo ligand exchange because it is a complex ion. In conclusion, the capability of the magnesium and strontium ions to be effective cofactors hinges on the ions' ability to exchange ligands with the phosphate backbone of the nucleic acids.

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