Antibiotic Resistance in Bacteria: Novel Metalloenzyme Inhibitors

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(Running Title: Metallo-ß-lactamase Inhibitors)

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Abstract

ß-Lactam antibiotics are among the most important drugs used to fight bacterial infection. Overuse and misuse of ß-lactam antibiotics has caused the evolution of resistance mechanisms, allowing pathogenic bacteria to survive antibiotic treatment. The major source of resistance to ß-lactam antibiotics occurs through production of enzymes called ß-lactamases capable of catalyzing hydrolysis of the ß-lactam rings in these drug compounds. The metallo-ß-lactamases have become a major threat due to their broad substrate specificities; there are no clinically useful inhibitors for these metalloenzymes. We have obtained single-stranded DNA's that are potent inhibitors of the *Bacillus cereus* 5/B/6 metallo-ß-lactamase. These are rapid, reversible, noncompetitive inhibitors of the metalloenzyme, with K_i and K_i' values in the nanomolar range. The inhibition patterns and metal ion dependence of their inhibition suggest that the oligonucleotides alter the coordination of the active site metal ion(s); inhibition is efficient and highly specific. Microbiological growth experiments, using combinations of ssDNA with the ß-lactam antibiotic cephalexin, reveal that the inhibitor is capable of causing cell death in liquid cultures of both Gram-positive and Gram-negative metallo - ß - lactamase - producing bacteria in the micromolar concentration range.

Introduction

ß-Lactam antibiotics such as penicillins, cephalosporins and carbapenems are widely used as antimicrobial drugs because they are relatively inexpensive and have been very effective (1). Pathogenic bacteria are becoming increasingly resistant to ß-lactam antibiotics. A common reason for this resistance is the inheritance of genes for the expression of efficient enzymes called ß-lactamases that catalyze the hydrolytic inactivation of the ß-lactam rings of the antibiotics. The metal-dependent metallo-ß-lactamases are the most problematic of these enzymes because of their wide range of substrate specificity and current lack of clinically useful ß-lactamase inhibitors (2). Study of such enzyme inhibitors is important clinically for the purposes of developing compounds that can be used in combination with existing ß-lactam antibiotics. We have utilized a combinatorial chemistry and *in vitro* screening technique called SELEX (Systematic Evolution of Ligands by EXponential Enrichment) (3, 4) to obtain potent single-stranded DNA inhibitors of the metallo-ß-lactamase from the organism *Bacillus cereus* 5/B/6. We report steady state enzyme kinetic and electronic spectral data that suggest that the single stranded DNA can rapidly, reversibly inhibit this metallo-ß-lactamase in a fashion that suggests that the inhibitors interfere with the active site metal ion(s) (5). Furthermore, combinations of the ß-lactam antibiotic cephalexin with the single stranded DNA can lead to a suppression of the growth of both Gram-positive (6) and Gram-negative bacteria on agar plates or in liquid cultures.

Key Words: Metallo-ß-lactamases, Inhibitors, Metalloenzymes, ß-Lactam antibiotics, Antibiotic resistance

Abbreviations: EXAFS: Extended X-ray absorption fine structure; EPR: electron paramagnetic resonance; LB: Luria-Bertani; MOPS: 3-(N-morpholino) propanesulfonic acid; PCI: Phenol:chloroform:isoamyl alcohol; PCR: polymerase chain reaction; ssDNA: single stranded DNA; TBE: tris borate EDTA; TEMED: tetramethylene diamine:

Methods and Materials

T4 DNA ligase was purchased from Promega, Madison, WI. Restriction endonucleases NdeI and SacI were purchased from New England Biolabs, Inc., Ipswich, MA and were used according to the manufacturer's recommendations. DNA molecular weight markers (BstNI digested pBR322 and BstEII digested λ DNA) were purchased from New England Biolabs, Inc. DEAE-Sephacel, Sephadex G-25 (superfine), CM-Sepharose CL 6B and various columns were purchased from Pharmacia. Uppsala, Sweden or Bio-Rad Laboratories, Hercules, CA. The Gene Clean II Kit was purchased from BIO101. Automated DNA sequencing was done on ABI, PRISMTM 310 Genetic Analyzer from Applied Biosystems Inc, Foster City, CA. Synthetic oligonucleotides for SELEX were synthesized using a Beckman Oligo 1000M oligonucleotide synthesizer. PCR reactions were carried out using a Perkin Elmer Certus Thermal Cycler. *Pfu* polymerase was purchased from Stratagene, La Jolla, CA. The cell suspensions were sonicated using a Heat System Ultrasonics, Inc., Farmingdale, NY, model W-375 sonicator. PCI (Phenol: Chloroform: Isoamyl alcohol (25:24:1) and electrophoresis grade agarose were obtained from Amresco, Solon, OH. Porcine carboxypeptidase A, hippuryl-L-phenylalanine, PCR 20 bp low ladder, ethidium bromide, dimethysulfoxide (DMSO), acrylamide, bisacrylamide, benzylpenicillin, cephalosporin C (potassium salt), ampicillin, cesium chloride, EDTA, ethanol, glucose, sodium hydroxide, potassium hydroxide, rubidium chloride, urea, MOPS, Tris, $ZnSO_4$ and various other inorganic salts and organic solvents of reagent grade or better were obtained from Sigma Chemical Company, St. Louis, MO. Difco, Franklin Lakes, NJ, brand bacto-agar, casamino acids and yeast extract used to make all media and plates were obtained through Fisher Scientific, Pittsburg, PA.

Enzyme Purification and Enzyme Assays

The metallo-ß-lactamase from *B. cereus* 5/B/6 was produced from *Escherichia coli* MZ1 harboring the pRE2/*bla* plasmid and purified according to the procedures described previously (7). The purity was ascertained by specific activity, native and SDS PAGE, and DE-MALDI-TOF. For metallo-ß-lactamase activity assays, the activities using cephalosporin C as substrate were determined as previously reported (8). The activity assay procedure was essentially the same as the spectrophotometric method of Davies et al. (9) in which the substrate absorbance at 260 nm is continuously monitored during hydrolysis. One unit of activity was defined as the amount of enzyme required to catalyze the hydrolysis of 1 μmol of substrate in one minute at 30 ºC. All assays were carried out near V_{max} using 4.3 mM cephalosporin C (approximately 10 K_m) dissolved in 50 mM MOPS/1 mM $ZnSO_4$, pH = 7.0. The assays were performed in a 0.1 cm pathlength quartz cuvette and the total reaction volume was maintained at 250 mL.

For ß-lactamase I activity assays, we used a modification of the method described by Davies et al. (9). The enzyme sample was incubated with 20 mM EDTA (pH = 7.0) for 15 min at room temperature prior to the assay. The enzymatic hydrolysis of 1.1 mM benzylpenicillin in 10 mM sodium citrate (pH = 7.0) and 1 mM EDTA was continuously monitored at 231 nm at 30 ºC. One unit of ß-lactamase activity was defined as the amount of enzyme required to hydrolyze one μmole of substrate in one minute at 30°C and pH = 7.0.

The assay of porcine carboxypeptidase A is based on the method of Folk and Schirmer (10). The rate of hydrolysis of hippuryl-L-phenylalanine is determined by monitoring the increase in absorbance at 254 nm (25 ºC, pH = 7.5). The enzyme was dissolved in 10 % lithium chloride to a concentration of 1 – 3 units per mL Hippuryl-L-phenylalanine (1 mM) was

dissolved in 0.05 M Tris HCl, pH = 7.5 with 0.5 M sodium chloride. In a 1 cm cuvette, 1.0 mL of substrate was added and incubated in the spectrophotometer at 25 ºC for 3 - 4 minutes to reach temperature equilibration and establish a blank rate. Fifty μL of diluted enzyme was added to initiate the reaction. The enzyme –was preincubated with or without the inhibitor in the buffer for 15 minutes at 25 ºC.

The protein concentrations were determined by the method of Lowry (11) using bovine serum albumin as a standard. This method was used throughout for all protein determinations.

SELEX

One 61 base single-stranded DNA was synthesized containing 30 bases of randomized sequence $(N)_{30}$ between two primer regions encompassing SacI and NdeI restriction endonuclease recognition sites respectively (underlined):

5'-CGCGAGCTCCGCGCG$(N)_{30}$CGCGCGCATATGGCGC-3'
Sac I Nde I

This template DNA was amplified by PCR with the corresponding primers, a 5' Primer (16 residues) possessing a NdeI site:

5'GCGCCATATGCGCGCG3'

and a 3' Primer (15 residues) possessing a SacI site:

5'CGCGAGCTCCGCGCG3'

The purified stock enzyme in 150 mM ammonium sulfate, 10 mM sodium citrate (pH = 7.0), 1 mM $ZnSO_4$, and 30 % (v/v) glycerol, was heated for 30 min at 60 ºC to denature any possible trace contaminant proteins. This enzyme is stable at 60 ºC. The enzyme was centrifuged and the supernatant was collected. The enzyme was diluted with dilution buffer (20 mM TA (tris-acetate) and 1 mM $ZnSO_4$, pH = 7.0). The synthesized library of 61-mer ssDNA described above was used for SELEX selection. The ssDNA was incubated with the enzyme at 30 ºC for 15 min in TA buffer with an appropriate concentration of NaCl; the total reaction volume was 20 μL. The amounts of NaCl in the incubated buffer were adjusted from 10 – 50 mM. After 15 min, glycerol was added to each sample to give 10 % (v/v) glycerol as a final concentration. Samples were run in the 6 % (w/v) polyacrylamide gel at 200 V for 25 to 30 minutes. The SELEX electrophoretic mobility shift assay used 6 % (w/v) polyacrylamide gels (29:1 monoacrylamide:bis-acrylamide) in 20 mM TA buffer (pH = 7.0), polymerized with 0.07 % (w/v) ammonium persulfate and 0.028 % (v/v) TEMED.

The enzyme:ssDNA complexes were separated from unbound DNA on the 6 % (w/v) polyacrylamide gels. The resulting gels were soaked in the incubation buffer with ethidium bromide for 10 minutes and destained in distilled, deionized H_2O. The enzyme:ssDNA complexes were visualized by UV illumination using TM-36 Chromato-UVE transilluminator from UVP Inc. The bands containing bound DNA were excised from the gel and crushed by a disposable pipette tip in the PCR tube. The ssDNA was amplified with 2.5 units of the *pfu* polymerase. The reaction mixture, including 200 ng of 5' primer (16 residues) and 100 ng of 3'primer (15 residues), was subjected to 30 cycles of 45 seconds at 94 ºC, 45 seconds at 55 ºC,

and 6 seconds at 72 ºC. This was followed by ten minutes at 72 ºC to allow all annealed primers to finish extending. The optimal 10x buffer for PCR was 100 mM Tris-HCl (pH = 8.8), 35 mM $MgCl_2$ and 250 mM KCl. The final concentration of dNTP was 2 mM. The total reaction volume was 100 μL.

The PCR products were purified from 12 % (w/v) polyacrylamide gel (29:1 monoacrylamide:bis-acrylamide). After cutting out the segment of the gel using a sharp scalpel or razor blade, the slice was transferred to a microcentrifuge tube. The slice was crushed using a disposable pipette tip. The slice was weighed to determine its volume and 1-2 volumes of elution buffer (0.5 M ammonium acetate, 1 mM EDTA (pH = 8.0), and 0.1 % (w/v) SDS) was added. The tube was incubated at 45 ºC on a rotary platform for 2.5 - 3 hours. After centrifuging the tube at 12,000 g for 1 minute, the supernatant was transferred to a fresh microcentrifuge tube. To avoid any fragments of polyacrylamide, a plastic column containing glass wool was used to centrifuge the supernatant. A one-half volume aliquot of elution buffer was added to the remaining pellet, mixed and recentrifuged. The supernatant and gel fragments were poured into the plastic column and spun for 15 seconds; 2-2.5 volumes of 100 % ethanol was added to the sample from this column and placed at –20 ºC for 1 hour and at –80 ºC for 10–15 minutes. The tube was spun for 10-15 minutes. This ethanol precipitation step helps in the removal of ethidium bromide. The supernatant was discarded. The pellet was washed with 70 % ethanol and was dried.

To confirm that the PCR product was the same size as the original ssDNA containing 30 random bases the two samples were compared on 12 % (w/v) polyacrylamide (29:1 monoacrylamide:bis-acrylamide) and 8 M urea gel in TBE buffer (12).

The plasmid pRE2/*bla* was digested with restriction endonucleases NdeI and SacI (13). All these double-digestion mixtures were electrophoretically separated on 1.0 % (w/v) agarose gel in TBE buffer at 60 V in the absence of ethidium bromide for 3 hours. The linear pRE2 vector and the metallo-ß-lactamase gene fragment were then located by staining the gels in a 5 mg/mL ethidium bromide solution and visualized under UV. The restricted linear pRE2 was then excised from the gels, and extracted by the Gene Clean Kit.

The ssDNA was amplified by PCR to make double stranded DNA (dsDNA). After ethanol precipitation, the fixed regions was digested with restriction endonuclease NdeI and SacI. The digested fragment was loaded on 12% (w/v) polyacrylamide gels (29:1 monoacrylamide:bisacrylamide) and was then purified by the crush and soak method. Ligation of the fragments with the linear pRE2 vector was accomplished with T4 DNA ligase at 4 ºC overnight or at room temperature for 3 hours. For each ligation, 100 ng of linearized pRE2 vector, 1.11 ng of fragment and 3 units of T4 DNA ligase were mixed together in ligation buffer in a total volume of 10 mL. After incubation, the mixture was used to transform *E. coli* strain TAP 56 competent cell prepared by the Hanahan method (14). Transformed cells were incubated at 30 ºC for 2 - 5 hours and were then put into LB medium that contained 1.0 % (w/v) casamino acids, 0.5 % (w/v) yeast extract, 0.5 % (w/v) sodium chloride (adjusted to pH = 7.0 with NaOH) and 50 mg/mL ampicillin. The culture was incubated at 30 ºC overnight. The subcloned plasmid DNA was prepared by the boiling miniprep method (12), and sequenced as described above. Twenty-one cycles of SELEX used to obtain the single sequence; from the seventeenth cycle to the twenty-first cycle, the time period of the incubation of ssDNA and enzyme was 2.5 hours. This 30-residue ssDNA synthesized as described above and subsequently purified by electrophoresis on a 12 % (w/v) polyacrylamide gel for all further experiments. The HPLC - purified 10-residue ssDNA was purchased from the Oligo Factory (Holliston, MA).

Bacterial Growth Experiments

For the microbiological experiments, *B. cereus* 5/B/6 was grown at 30° C in "S" broth (8) while *E. co*li transformed with the expression vector plasmid PRE2*/bla* was grown at the enzyme induction temperature (42°C) in LB medium.

Results and Discussion

The SELEX method is used to screen for nucleic acid aptamers. Aptamers are oligonucleotide or peptide molecules that have the ability to bind to specific target molecules in a molecular recognition fashion that rivals antibodies (15). While it may at first seem surprising that a bacterial enzyme that presumably has no contact with nucleic acids in cells during its normal function can be so efficiently and specifically inhibited by nucleic acid aptamers, we have previously observed a strong association of the crude enzyme with nucleic acids during early stages of the metallo-ß-lactamase purification scheme (7).

Figure 1 shows two photographs of the identical polyacrylamide electrophoresis gel containing a mixture of single-stranded DNA bound to the purified metallo-ß-lactamase during the first cycle of SELEX. On the left, the gel was soaked in an ethidium bromide solution and illuminated to reveal the bound single-stranded DNA. On the right, the same gel was stained with a protein specific stain, coomaisse blue. Clearly, both the metallo-ß-lactamase and DNA are present in this band. Under the conditions of this experiment, the metallo-ß-lactamase is cationic and the anode is placed at the bottom of the gel. Normally, the enzyme would not even enter the gel, but because it is tightly bound to the anionic DNA present, the complex migrates toward the gel bottom. Unbound DNA is not visible here because it has run off the bottom of the gel.

After 21 cycles of SELEX, the final 30 residue DNA sequence that was obtained was:

5'-d(AACCAAACTTGGATCGGTGCACATGTCGAA)-3'

Figure 2 shows the steady state enzyme kinetic data of the inhibition of the *B. cereus* 5/B/6 metallo-ß-lactamase by various concentrations of the 30 residue single-stranded DNA. The Lineweaver-Burk plot shown in Figure 2 is typical for rapid, reversible, noncompetitive inhibition. Indeed, a similar kinetic pattern was obtained for inhibition of the metallo-ß-lactamase by the divalent metal chelating compound EDTA (data not shown). Slope and intercept replots of the primary data revealed the dissociation constants for the enzyme-inhibitor complex (K_i) and the Michaelis complex (K_i') respectively and their values are listed in Table I. Clearly, with all the inhibition constants in the nanomolar range, inhibition of the metallo-ß-lactamase by the 30 residue DNA is quite effective. By comparison, the K_i value for EDTA was 3 μM.

An analysis of the 30 residue single stranded DNA sequence for possible segments of expected secondary structure by the M-fold program (16) revealed the predicted 10 residue segment depicted diagrammatically in Figure 3. When this 10 residue DNA was synthesized, this 10 residue stretch was found to also inhibit the metallo-ß-lactamase in a noncompetitive fashion as was true for the 30 residue sequence (Figure 2). In fact, all of the inhibition was associated with this 10 residue sequence. When we synthesized the 9 residue sequence upstream and the 10 residue sequence downstream of the sequence in Figure 3, neither of these single stranded DNA's had any effect at all on the metallo-ß-lactamase activity (data not shown). Further analysis of the kinetic inhibition pattern for the 10 residue DNA yield the constants listed in Table I; inhibition by the 10 residue DNA is very similar to that by the 30 residue DNA. The rest of our experiments were performed using the 10 residue single stranded DNA shown in Figure 3.

Since inhibition of metalloenzymes by metal chelators is expected to be noncompetitive, we tested the 10 residue DNA as a reversible inhibitor of a different, typical zinc-dependent enzyme, porcine carboxypeptidase A. Even at 25 times the concentration of the K_i for the metallo-ß-lactamase, the DNA had no effect on the enzymatic activity of porcine carboxypeptidase. Hence, the DNA does not indiscriminately chelate the active site zinc but rather is highly specific for the *B. cereus* 5/B/6 metallo-ß-lactamase to which it obviously binds tightly. Furthermore, a concentration of the 10 residue DNA 25 times the K_i for the metallo-ß-lactamase had no effect on the enzymatic activity of another, metal-independent ß-lactamase. Clearly, even though benzylpenicillin is a very good substrate for both enzymes (hence their substrate – binding domains have some similarities) the DNA has no effect whatsoever on the activity of the *B. cereus* 569/H/9 metal-independent ß-lactamase I. Clearly, the DNA is not competing for the active site. Still further support for the idea that the inhibition involves the active site metal(s) comes from the fact that when the inhibition is carried in excess zinc (1 mM) the inhibitor is six-fold less effective than in the absence of exogenous zinc (data not shown).

We conclude that not only does the DNA bind the metallo-ß-lactamase tightly, but that it does so by interfering with the coordination of one or more active site zinc ions by binding closely to the active site metal(s) and/or perhaps by coordinating one or more of the active site metals. Either or both of these ideas are consistent with noncompetitive inhibition patterns observed in the presence of the 10 or 30 residue DNA's; neither inhibitor simply competes for the substrate binding site. To completely evaluate the possibility of tight-binding inhibition of the enzyme by the DNA, a more extensive analysis of the inhibition of the enzyme by the DNA at various enzyme concentrations will be required.

It is possible to prepare the apoenzyme of the metallo-ß-lactamase and reconstitute the some of the original enzymatic activity by the addition of cobalt (II) sulfate (18). Figure 4 shows the visible electronic spectra of the Co (II)-reconstituted metallo-ß-lactamase in the absence (dotted line) and presence (solid line) of an excess of the 10 residue DNA. The changes in the spectrum indicate a change in the coordination sphere of the active site metal and are thought to be associated with a perturbation of the charge transfer band from the metal to the only cysteine thiol in the protein. Similar changes, of somewhat larger magnitude, are observed in the presence of an excess of substrates such as cephalosporin C (17, 18). Thus, we have spectral evidence that the aptamer binds tightly to the enzyme in a fashion consistent with our kinetic data at or near the metal binding sites. The K_i and K_i' data of Table I show that the 10 residue DNA can also inhibit the cobalt-reconstituted form of the enzyme and serves to further demonstrate the exquisite specificity of the DNA aptamer in that the DNA is able to distinguish between the zinc and cobalt forms of the enzyme even though both enzyme forms possess the same enzymatic activity at the same active site.

Figure 5 A and B demonstrates that the combination of the 10 residue DNA aptamer with a typical ß-lactam antibiotic (cephalexin) can cause cell death over a period of several hours of both Gram-positive and Gram-negative antibiotic resistant bacteria. In Figure 5A, the combination of DNA and antibiotic suppresses Gram-positive *B. cereus* 5/B/6 growth in liquid culture with only a single dose of DNA over a 20 hour period at 30 °C. Note that the control cultures with either antibiotic alone or inhibitor alone do not cause growth suppression. In the case of Gram-negative *E. coli* TAP 56 in Figure 5B, the growth was done at 42 °C in order to induce the *B. cereus* metallo-ß-lactamase in the *E. coli* cells transformed with pRE2/*bla*. Clearly under the conditions of the latter experiment, the DNA was easily able to enter the intact *E. coli* cells; the 10 residue DNA has a molar mass of 3 kDA. From separate studies of the growth of

B. cereus and *E. coli* at various concentrations of the 10 residue DNA (data not shown), we were able to determine for each bacterium a concentration of the DNA that results in 50 percent cell death (LC_{50}) in their respective liquid cultures. In the presence of 5 μM cephalexin, the LC_{50} values obtained for *B. cereus* 5/B/6 and *E. coli* TAP56 were 75 and 32 μM respectively. Clearly, these LC_{50} values are higher than the K_i and K_i' values obtained for purified enzyme. This might be a result of possible exonuclease activity in the bacterial cultures or perhaps the endogenous zinc ion concentration in the culture media. Putative exonuclease activity might also explain why the E. coli culture of Figure 5Beventually begins to grow again.

Conclusions and Future Directions

We have shown that single-stranded DNA aptamers can be highly specific and effective inhibitors of the *B. cereus* 5/B/6 metallo-ß-lactamase both in Gram-positive and Gram-negative antibiotic resistant bacteria. They appear to act by specifically interfering with the active site metal ions of the enzyme.

Our continuing investigations of these inhibitors involve testing their efficacy with other bacterial metallo-ß-lactamases. Likewise, we are also investigating bacterial metallo-ß-lactamase inhibition with other nucleic acid aptamers including double-stranded DNA and RNA (15); such studies may provide further insight into the details of the mechanism of the inhibition. Whether or not such aptamers can be used as they are as *in vivo* enzyme inhibitors is also being investigated. Even if the latter goal cannot be realized, nucleic acid aptamers provide an intriguing new lead to the inhibition of metallo-ß-lactamases that may result in the development of new small molecule inhibitors. As a result, we are actively pursuing structural data for the inhibitors and inhibited enzyme complexes using X-ray diffraction, EXAFS, EPR and NMR.

Acknowledgements

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Figure Legends

Figure 1. The evidence for a complex of the *B. cereus* 5/B/6 metallo-β-lactamase and ssDNA. On the left, the gel was stained by ethidium bromide. On the right, the gel was stained by Coomassie Brilliant Blue R250. 20 μM enzyme and 1.5 μM ssDNA were used to make the complex. The buffer used for incubation was 20 mM TA (pH = 7.0) and 1 mM $ZnSO_4$.

Figure 2. Lineweaver-Burk plot of inhibition of *B. cereus* 5/B/6 metallo-β-lactamase by the 30 residue single stranded DNA. For the filled circles: [DNA] = 0 nM; open circle: [DNA] = 1 nM; filled square: [DNA] = 2 nM; open square: [DNA] = 3 nM. Steady state kinetic assays were performed as described in Methods and Materials.

Figure 3. Diagrammatic representation of the predicted secondary structure for the 10 residue single stranded DNA sequence derived from the longer 30 residue sequence. The dots are intended to represent hydrogen-bonded base pairs.

Figure 4. Electronic absorbance spectra of 0.7 mM *Bacillus cereus* 5/B/6 metallo-ß-lactamase in 50 mM MOPS/ 1mM Co (II) sulfate, pH= 7.0 in the absence (dotted line) and presence (solid line) of 1.6 mM ssDNA. The path length was 1 cm.

Figure 5. Panel A. *B. cereus* growth in liquid cultures. Three 1 mL cultures of "S" broth were inoculated with *B. cereus* 5/B/6 cells and shaken for 20 hours at 30° C. The blue circles represent the control with only the *B. cereus*. The green circles represent the *B. cereus* with 1 μM cephalexin (antibiotic). The circles represent growth of *B. cereus* with 1 μM cephalexin and 0.3 mM 10 residue DNA.

Panel B. *E. coli* growth in liquid cultures. Two 1 mL cultures of LB broth were inoculated with *E. coli* TAP 56 cells transformed with our metallo-ß-lactamase expression vector plasmid and shaken for 10 hours at 42 °C. The diamonds represent growth of *E. coli* in LB broth with 5 μM cephalexin and the circles represent growth of the *E. coli* in 5 μM cephalexin and 0.3 mM 10 residue DNA.

Figure 1

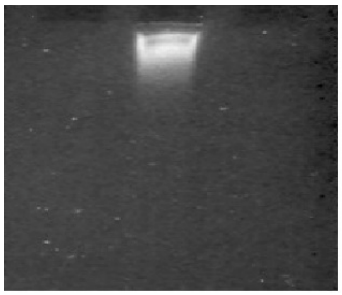


Figure 2

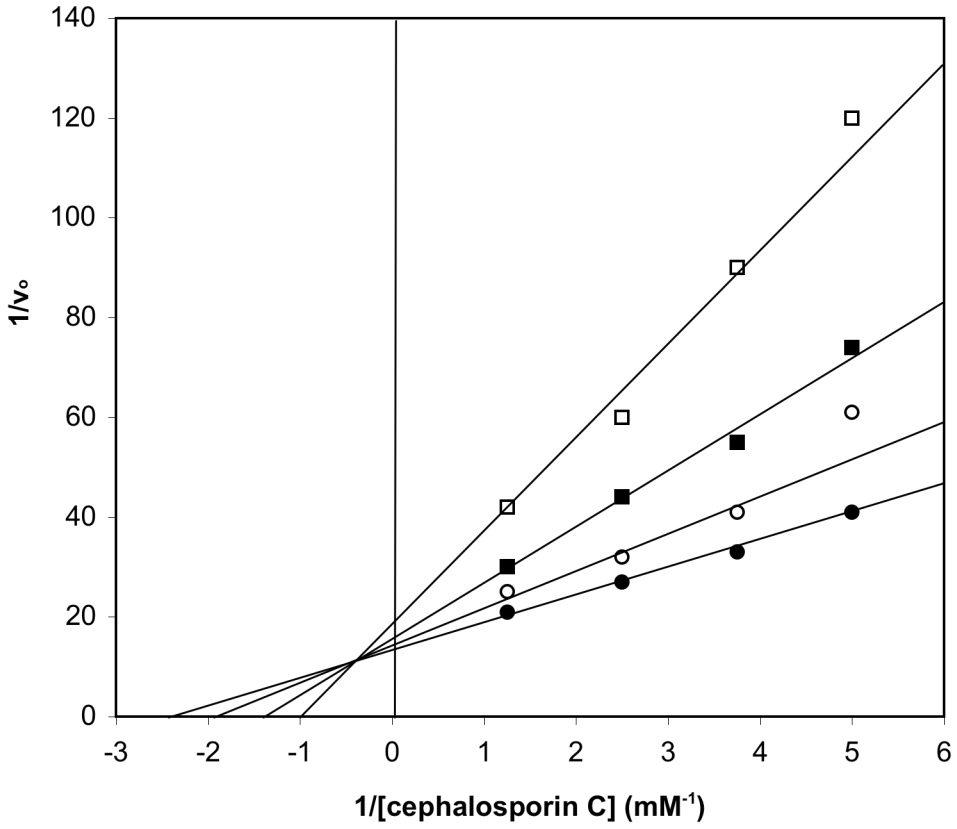


Figure 3

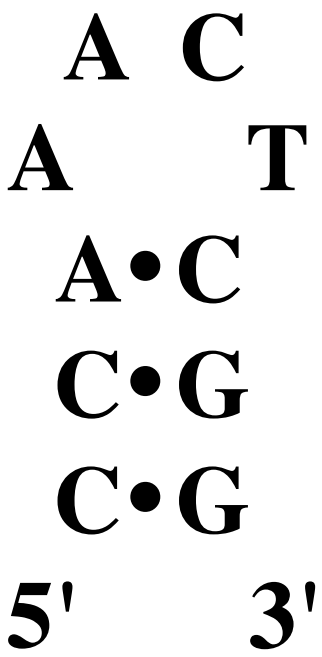
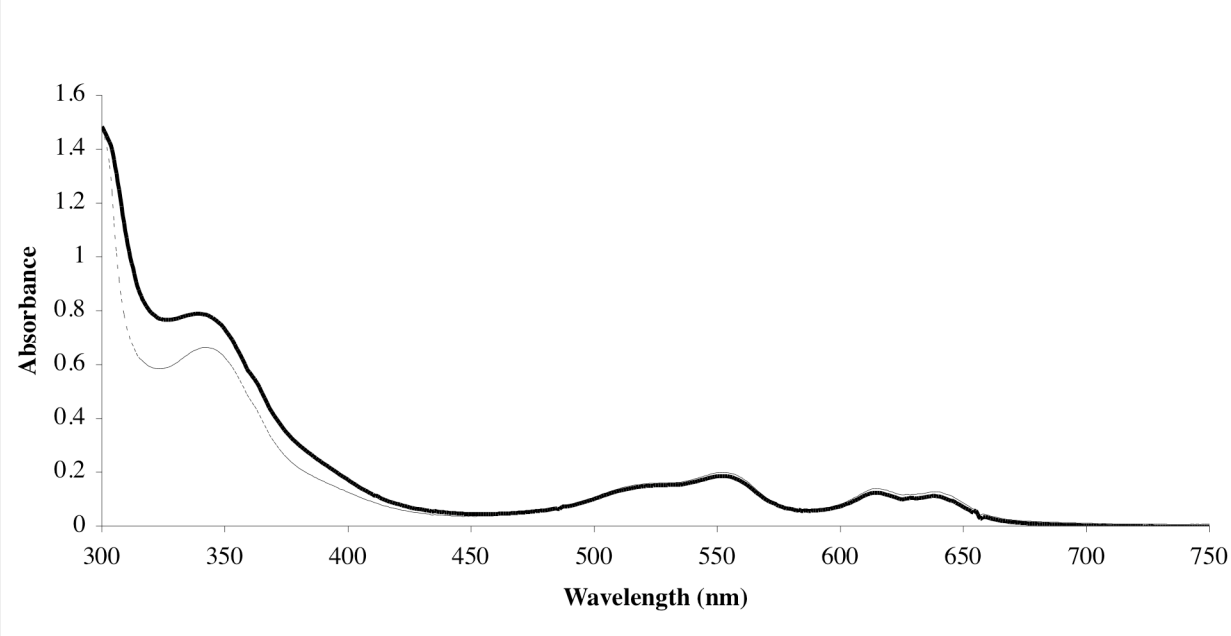


Figure 4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1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
300
350
400
450
500
550
600
650
700
750
Wavelength (nm)

Figure 5A

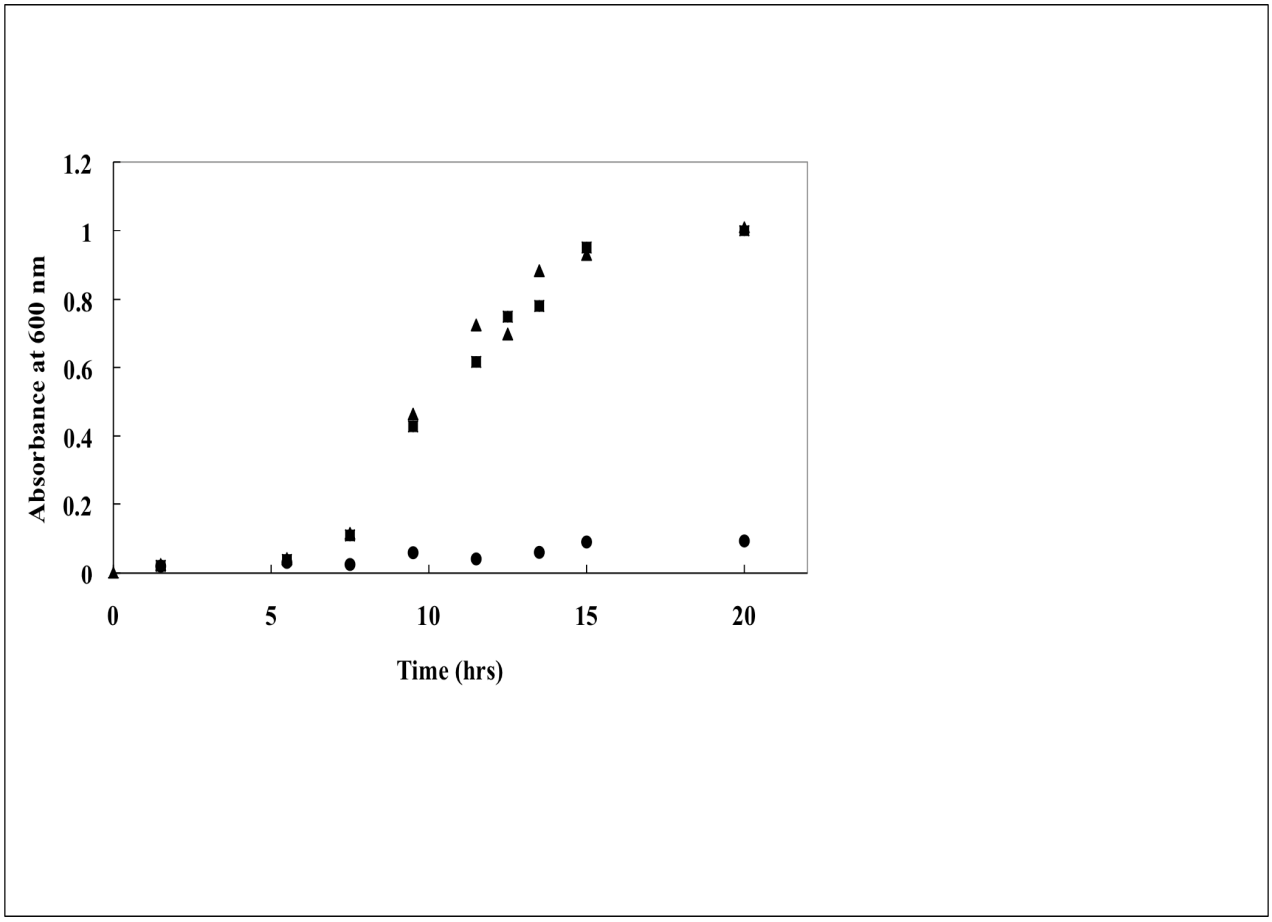

Figure 5B

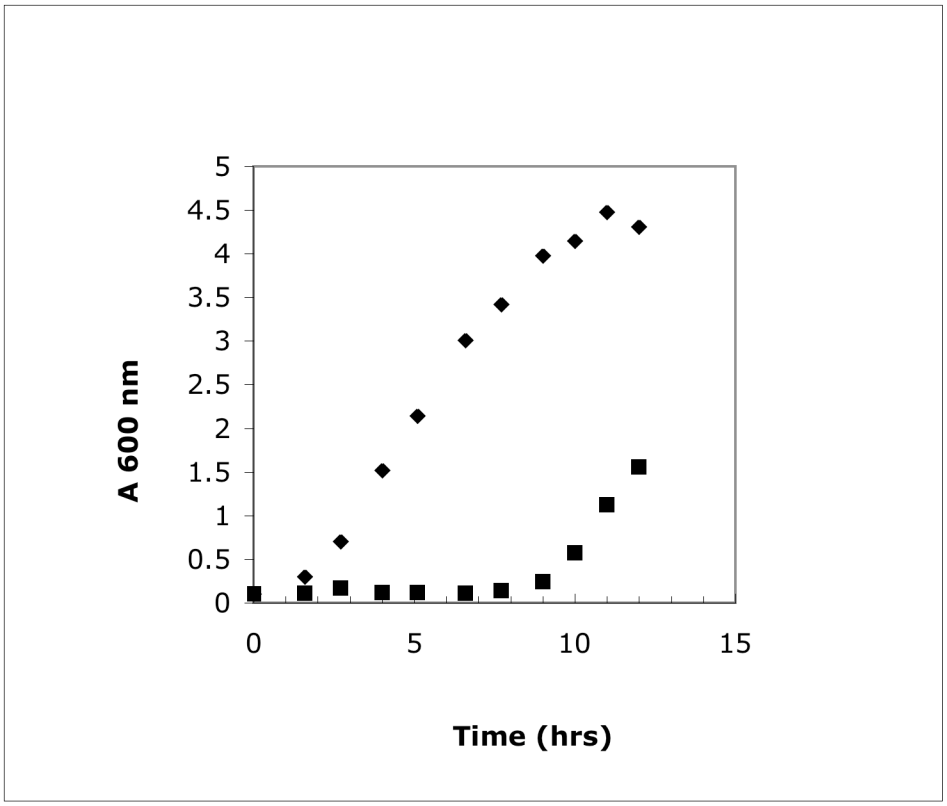

Table I. Steady State Kinetic Parameters for Inhibition of *Bacillus cereus* 5/B/6 Metallo-ß-lactamase by Single Stranded DNA

Aptamer	Enzyme Form	K_i (nM)	K_i' (nM)
30 residue ssDNA	zinc	0.92	11
10 residue ssDNA	zinc	0.31	1.5
10 residue ssDNA	cobalt	2.5×10^3	2.9×10^5