EXPRESSION, PURIFICATION, AND CHARACTERIZATION OF A POLYSACCHARIDE DEPOLYMERASE FROM *ACINETOBACTER BAUMANNII* BACTERIOPHAGE ABAUYA1

An Honors Fellow Thesis

by

DAVID MATTHEW MIGL

Submitted to Honors and Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

HONORS UNDERGRADUATE RESEARCH FELLOW

April 2012

Major: Molecular and Cell Biology

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Approved by:

Research Advisor: Ryland Young
Associate Director, Honors and Undergraduate Research: Duncan MacKenzie

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ABSTRACT

Expression, Purification, and Characterization of a Polysaccharide Depolymerase from *Acinetobacter baumannii* Bacteriophage AbauYa1. (May 2012)

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The use of bacteriophages offers an appealing alternative to antibiotics for the control of pathogenic bacteria. Recently, bacteriophage AbauYa1 was isolated as part of an effort to find phages to combat *Acinetobacter baumannii* infections. In addition to causing lysis of the host cell, AbauYa1 depolymerizes the polysaccharides of the *A. baumannii* capsules. The *A. baumannii* capsule is an important virulence factor, and phage depolymerases have been shown to disrupt biofilms of other pathogenic bacterial strains. The gene encoding the protein responsible for this activity was cloned, and the protein was expressed, purified, and enzymatically assayed. The protein was found to degrade the polysaccharide capsule, as shown by an increase in reducing ends upon incubating the capsule with the depolymerase. Finally, the protein removes *A. baumannii* biofilms from a polystyrene surface. The protein degrades *A. baumannii* capsule and biofilms and therefore carries high therapeutic potential for treating *A. baumannii* infections.

ACKNOWLEDGMENTS

I thank Dr. Jason Gill for serving as my primary mentor and instructor, Dr. Thammajun Wood for the plasmids containing gene *38* and initial work on gp38, Samir Moussa for assistance with protein purification and size exclusion chromatography, Dr. Ryland Young for serving as PI and reviewing the final manuscript, and all members of the Young lab for support and helpful discussions.

NOMENCLATURE

BCA	Bicinchoninc acid
CV	Crystal violet
EDTA	Ethylenediaminetetraacetic acid
EPS	Exopolysaccharide
His-tag	Hexahistidine tag
IPTG	Isopropyl β-D-1-thiogalactopyranoside
LB	Luria broth
PAGE	Polyacrylamide gel electrophoresis
SDS	Sodium dodecyl sulfate
Tricine	*N*-[2-hydroxy-1,1-bis(hydroxy-methyl)ethyl]glycine
TSA	Tryptic soy agar
TSB	Trypic soy broth
UV	Ultraviolet

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CHAPTER I

INTRODUCTION

Background

Acinetobacter baumannii as a pathogen

Acinetobacter baumannii is a Gram-negative, opportunistic pathogen notorious for contributing to multidrug-resistant battlefield-acquired and nosocomial infections (1). Multidrug-resistant bacteria are a pressing clinical issue, and yet few new antibiotics are being produced (2). Alternative strategies are needed for the control of pathogenic bacteria, and the use of bacteriophages offers an appealing alternative to small-molecule antibiotics for controlling bacterial infections (3). Phage therapy is built on the premise that bacteriophages could be therapeutic by causing lysis of a sufficient percentage of host cells (3). Additionally, *A. baumannii* produces an exopolysaccharide (EPS) capsule that contributes to biofilm formation (4) and is essential for virulence and survival in mammalian tissues (5). Thus, disruption of the capsular polysaccharide of *A. baumannii* would also be a valuable therapeutic strategy for combating *A. baumannii* infections. The bacteriophage AbaYau1 was recently isolated as part of an effort to discover novel phages with therapeutic potential against *A. baumannii*.

This thesis follows the style and format of *The Journal of Biological Chemistry*.

Prior work on AbauYa1

The plaque morphology of AbauYa1 suggested that a virion-associated protein possessed polysaccharide depolymerase activity. When the phage was plated on lawns of *A. baumannii*, clear plaques surrounded by an expanding turbid halo were produced, characteristics indicative of depolymerase activity (6). The clear central region of the plaque represents the area where the phage has lysed all the host bacteria, and the turbid halo corresponds to clearing of the bacterial capsule, but not lysis of the cells. Here "depolymerase activity" means any activity that cleaves polysaccharide chains into smaller subunits, regardless of the precise catalytic mechanism.

AbauYa1 was determined to belong to the *Podoviridae* by electron microscopy, possessing a short, non-contractile tail. In order to further characterize AbauYa1, the phage genome was sequenced and annotated, revealing a genome of 40,352 bp and 43 putative genes. AbauYa1 was determined to be a member of the T7 phage superfamily based on its genome organization. Interestingly, several T7-like podophages, including podophage K5, have been found to possess polysaccharide depolymerase activity as part of their tail assemblies (7).

Identification of the polysaccharide depolymerase gene

For these reasons it was hypothesized that a putative tail fiber gene in AbauYa1 was responsible for the observed putative depolymerase activity. Gene product 38 (gp38) from AbauYa1 was selected as the most likely candidate due to its protein sequence

similarity to other known depolymerases and the lack of other candidates in the AbauYa1 genome. Gene *38*, encoding the predicted depolymerase, was cloned, expressed, and partially purified. The resulting protein extract was able to generate reducing ends when incubated with purified *A. baumannii* EPS. Thus, the tail spike gp38 was concluded to be a highly likely candidate responsible for the depolymerase activity of the bacteriophage.

Rationale of the current project

Like all virulent phages, AbauYa1 destroys its host as part of its life cycle. However, the depolymerase activity represents a second mode of action whereby AbauYa1 attacks populations of its host. The depolymerase destroys the bacterial capsule and may also disrupt bacterial biofilms, major contributors to antibiotic resistance in some bacteria (8). The objective of this project is to confirm that gp38 is a polysaccharide depolymerase and use the depolymerase to degrade biofilms. Additionally, the depolymerase and its substrate, *A. baumannii* EPS, are almost wholly uncharacterized, so basic biochemical information about these two entities will be gathered. These studies will further the development of AbauYa1 gp38 as a potential treatment for drug-resistant *A. baumannii* infections.

CHAPTER II

METHODS

Strain construction

The bacterial strains used in this study are shown in Table 1. *Acinetobacter baumannii* strain AU0783 and *Escherichia coli* BL21(DE3) strains 30019 and 30026 carrying phage AbauYa1 *gp38* in a pET28B vector (EMD Biosciences) were obtained from Dr. Thammajun Wood (Texas A&M University). These were used to construct strains in *E. coli* BL21(DE3) *slyD* cells (strains #30030-30035). Plasmid DNA from strains 30019 and 30026 was isolated with the Qiagen QIAprep Spin Miniprep Kit (Qiagen) according to the manufacturer's protocol and transformed into strain 30030 cells that had been rendered chemically competent by the method of Chung et. al. (9). After recovery in SOC media (NEB) for 1h, transformants were selected on LB agar media containing 30 μg mL^{-1} kanamycin.

The sequence of all strains was confirmed by automated fluorescent sequencing by Eton Bioscience, Inc. (San Diego, CA). Frozen permanent cultures of all strains were created by combining 800 μL of a liquid overnight culture from a single colony picked from a fresh plate with 200 μL glycerol-saline solution and stored at −80 °C.

TABLE 1
Strains used in this study.

Strain number	Name	Host	Vector	Insert	Location of oligohistidine tag (NH$_3$/COOH terminus)
30002	AU0783	*A. baumannii* AU0783	---	---	---
30014	Iraq 1883	*A. baumannii* Iraq 1883	---	---	---
30019	---	*E. coli* BL21(DE3)	pET28B	AbauYa1 gp38	C
30022	Host	*E. coli* BL21(DE3) s*lyD*	None	---	---
30023	Control	*E. coli* BL21(DE3) s*lyD*	pET28B	None	---
30026	---	*E. coli* BL21(DE3)	pET28B	AbauYa1 gp38	N
30030	C-term	*E. coli* BL21(DE3) s*lyD*	pET28B	AbauYa1 gp38	C
30031	N-term	*E. coli* BL21(DE3) s*lyD*	pET28B	AbauYa1 gp38	N

Protein expression and purification

Starter cultures from a single colony from a freshly made plate were incubated in liquid LB medium with 30 μg mL^{-1} kanamycin overnight at 37 °C in a roller drum. These cultures were diluted 1:100 and grown to mid-log phase (A_{550} = 0.5) at 37 °C with aeration. Cultures were induced for 2 hours at 30 °C by the addition of isopropyl β-D-thiogalactoside (IPTG) to a final concentration of 1 mM. The A_{550} of cells was measured before collection by centrifugation at 10,000 g for 10 minutes.

Cells were resuspended at an equivalent OD_{550} of 30. Sigma protease inhibitor cocktail, and RNase and DNase (100 μg mL^{-1}) were added to the resuspension, and cells were ruptured by passage through a French pressure cell at 16000 psig. Cell debris was removed by centrifugation at 10,000 g for 10 minutes at 4 °C. The protein was purified by passing the supernatant over a Talon metal affinity resin (Clontech). The column was washed with 10 bed volumes of wash buffer (50mM phosphate [pH 7.2] and 300 mM

NaCl) and eluted in two bed volumes of wash buffer supplemented with 400 mM imidazole. Samples were dialyzed three times against 3500 Da molecular weight cutoff filter (Thermo Scientific) and 1X assay buffer (25 mM Tris-hydrochloride [pH 7.2] and 150 mM NaCl) (10^3 volumes assay buffer per 1 volume sample).

Size exclusion chromatography was performed on a Superdex 200 size exclusion column (GE Healthcare; 10 x 300 mm, 24 mL bed volume, equilibrated with 1X assay buffer) and an Akta FPLC system at room temperature with a flow rate of 0.5 mL assay buffer min^{-1}, collected in 0.5 mL fractions.

***A. baumannii* exopolysaccharide (EPS) purification**

A. baumannii strain AU0783 was grown to confluence on tryptic soy agar plates supplemented with 0.5% (w/v) glucose and incubated at 37 °C. Incubation times were varied from 72h to 120h to test the effects of incubation time on EPS production. EPS purification was carried out by the method of Steinmetz et. al. (10). Briefly, cells were harvested by scraping them from the agar surface with 10 ml of 0.9% (w/v) NaCl per plate, adding 5% (v/v) phenol, and agitating with a stir bar and stir plate for six hours. Cells were cleared by centrifugation at 10,000 g for 10 minutes and the supernatant was precipitated with five volumes of 95% ethanol. The precipitated EPS was resuspended in water and digested with DNase and RNase (1 μg mL^{-1}) at 37 °C for one hour. The digest was stopped by heat inactivation at 65°C for 10 minutes. The EPS was freeze-dried with

a vacuum purification system, and the resulting solid EPS was weighed. EPS was resuspended at 5 μg ml^{-1} for depolymerase activity assays.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

2X SDS sample buffer (final: 125 mM Tris-hydrochloride pH 6.8, 4% SDS, 5% 2-mercaptoethanol, 10% glycerol) was added to each 10 μl aliquot and boiled in a water bath for 10 minutes. Gels were 10% polyacrylamide and used Tris-glycine as a buffer. Molecular weights were visualized by comparison with a marker (SeeBlue Plus2, Invitrogen). The gel was stained with Coomassie blue and destained overnight in a solution of 60% methanol, 10% acetic acid, and 30% water before imaging.

UV spectroscopy, protein concentration

The UV spectrum of the depolymerase was measured on a Hitachi U-0080D spectrophotometer. Protein extinction coefficients were calculated according to the method of Gill and von Hippel (11), and concentration was calculated using the expression $c = A \cdot \varepsilon^{-1} \cdot l^{-1}$.

EPS degradation assay

Depolymerase and EPS were incubated together at 37 °C in 25 mM Tris-hydrochloride (pH 7.2) and 150mM NaCl. The concentrations of the enzyme and EPS are noted under each relevant figure. For control experiments, the depolymerase was boiled for ten minutes in a water bath before being added to the rest of the reaction. For time-resolved

measurements, volumes of reagents were increased appropriately and aliquots were taken from the same tube, mixing between aliquots. The reaction was stopped by incubation at 100 °C for 10 minutes.

Bicinchoninic acid assay

Degradation of EPS by the depolymerase was assayed by measuring the amount of reducing ends in solution. The concentration of reducing ends was measured by the bicinchoninic acid (BCA) assay (12). Because the composition of the *A. baumannii* EPS is unknown, a glucose standard curve was generated every time measurements were made and absorbances were converted to glucose equivalent concentration of reducing ends. BCA solution A (final concentrations: 5.0 mM disodium 2,2'-bicinchoninate, 0.25 M sodium carbonate buffer pH 10.1) and solution B (5.0 mM $CuSO_4 \cdot 5H_2O$, 12 mM L-serine) were stored at -20 °C, and a working stock was made daily by mixing equal amounts of both reagents. 500 μL of the stock solution was added to a 25 μL aliquot of sample containing between 0-10 nmol equivalent of glucose reducing ends (0-400 μM), which is within the linear range of the Hitachi U-0080D spectrophotometer (A = 0.0 – 1.0) and well within the linear range of the assay (0.0 – 1.0 mM for a 25 μL aliquot). All samples were capped and boiled simultaneously in a water bath for 15 minutes and cooled at room temperature for at least 5 minutes before the A_{560} was measured.

Biofilm assay

Biofilm degradation assays were performed according to the method of Merritt *et. al.* (13). *A. baumannii* was grown in TSA liquid cultures overnight and diluted 1:100 into a total volume of 200 μL of LB in a 96-well polystyrene plate (Falcon microtest, #35-1172). After incubation at 37 °C for 48 h, the OD_{550} of the cells was read in a plate reader (Tecan). The culture medium and nonadherent cells were removed from the well and the remaining biofilm washed three times with 250 μL assay buffer (150 mM NaCl, 25 mM Tris pH 7.2). Depolymerase protein (0.2 μg in 200 μL assay buffer) was added to the wells receiving treatment and 200 μL assay buffer was added to the control wells. The plate was incubated without shaking at 37 °C for 9 h. To stain, the plate was washed three times with assay buffer, 150 μL crystal violet (CV) stain was added to each well, and the plate was left at room temperature for 15 minutes. The stain was removed, and wells were washed five times with distilled water. The plate was air dried for 15 minutes, and 200 μL of 30% acetic acid was added to each well to solubilize the remaining CV dye. 125 μL from each well was transferred to a new 96-well plate, and the A_{600} was read. Biofilm formation is reported as A_{600}/ OD_{550} to normalize for cell growth before depolymerase treatment.

CHAPTER III

RESULTS

Protein purification

Aliquots taken while purifying C-terminally his-tagged gp38 are shown in Figure 1. The final protein sample is >90% pure. Figure 2 shows the UV-Vis spectrum of gp38 used for quantifying the protein concentration. The spectrum exhibits a typical absorption peak at 280 nm and is free of contaminating nucleic acids, as evidenced by lack of a second peak at 260 nm. Table 2 gives the yield, molar mass, and extinction coefficient of the protein.

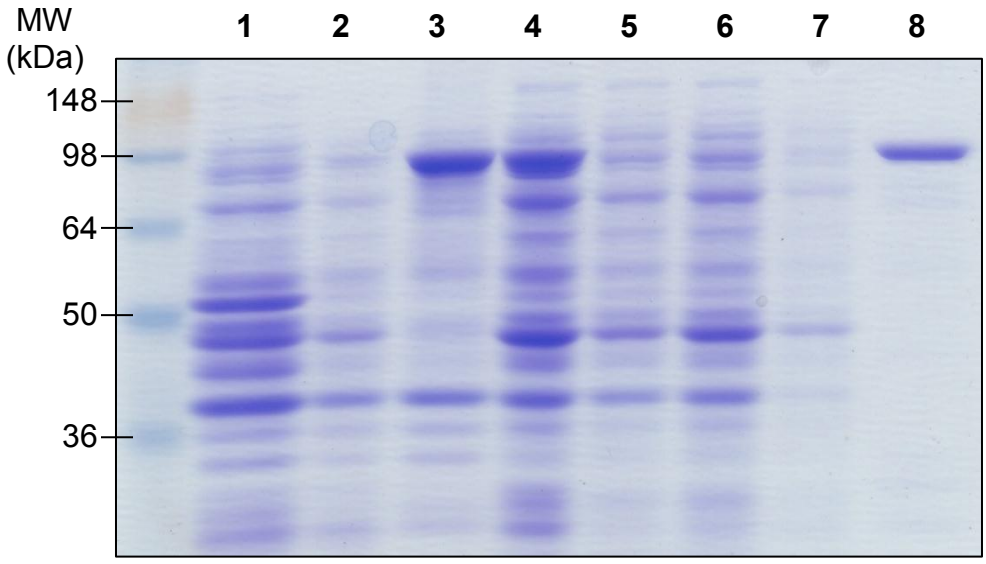

FIGURE 1. **Purification of the C-terminally His-tagged protein.** Aliquots from the purification process were electrophoresed on a 14% SDS-PAGE gel. Whole cell lysates (lane 1, control empty vector; lane 2, C-terminally his-tagged depolymerase) were disrupted by a French cell press and centrifuged to remove cell debris (supernatant, lane 3; pellet, lane 4). The oligohistidine tagged protein was purified by multiple passages over a metal affinity resin (loading flow through, lanes 5 and 6). The column was washed with 10 bed volumes of buffer (lane 7) and eluted in 400 mM imidazole (lane 8).

TABLE 2
Physical properties and purification results.

Molar mass	102.4 kDa
Extinction coefficient	140070 $cm^{-1}M^{-1}$
Volume of culture induced	50 mL
Mass of purified protein (mg)	1.90 mg
Yield (mg/L)	38 mg/L

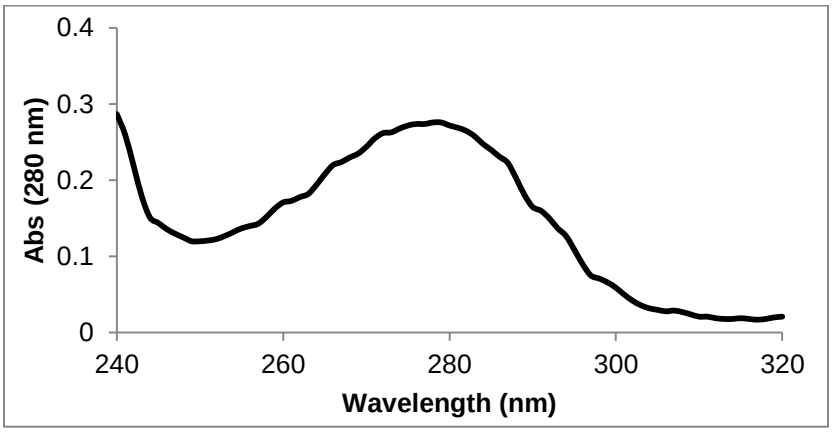

FIGURE 2. **UV-Vis spectrum of gp38.** The sample was read on a Hitachi U-0080D spectrophotometer (*l* of the cuvette = 0.5 cm).

Exopolysaccharide purification

Two separate EPS purifications were carried out. EPS was harvested at either 72 h or 120 h. Table 3 shows the different yields that resulted.

TABLE 3
Results of EPS purification.

Number of Plates	Incubation time (h)	EPS yield (mg)	Yield per plate (mg)
20	72	20.0	1.0
40	120	164.0	4.1

AbauYa1 gp38 is a polysaccharide depolymerase

The A. baumannii EPS chain terminates in a reducing sugar. If it is a depolymerase, AbauYa1 gp38 will generate additional reducing ends as it cleaves the EPS into smaller subunits. Figure 3 shows that C-terminally his-tagged gp38 degrades A. baumannii EPS, as evidenced by a fourfold increase in concentration of reducing ends upon incubation with the sugar at 37 °C for 1 h. The protein with the N-terminal hexahistidine tag did not exhibit similar activity. This lack of activity was not investigated, and C-terminally his-tagged gp38 (referred to as "the depolymerase") was used for all further experiments.

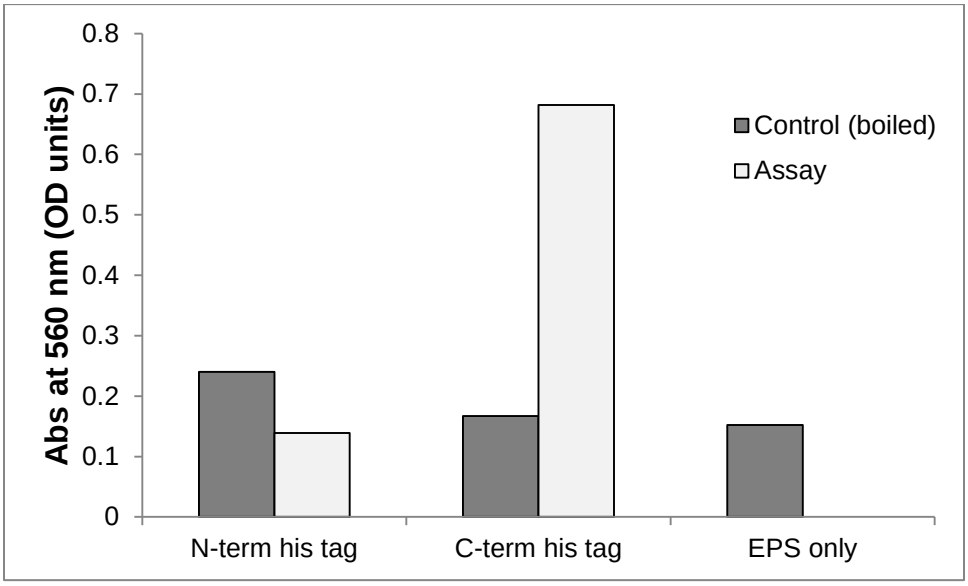

FIGURE 3**. Enzymatic activity displayed by purified proteins.** Depolymerase protein (final concentration: 0.2 µg/µl) was incubated with EPS (0.5 µg/µl) in 150 mM NaCl, 25 mM Tris pH 7.2 at 37 °C for 1 hour, and the concentration of reducing ends in each aliquot was measured by the BCA assay.

Figure 4 shows a kinetic trace of the reaction. The reaction in Figure 4 used less depolymerase than that in Figure 3, yet reaction was largely complete by 20 minutes. Extending the incubation time to 200 minutes did not result in any increase in reducing ends (data not shown). Therefore, the concentration of reducing ends in Figure 3 is near the maximum extent of reaction for the amount of substrate used.

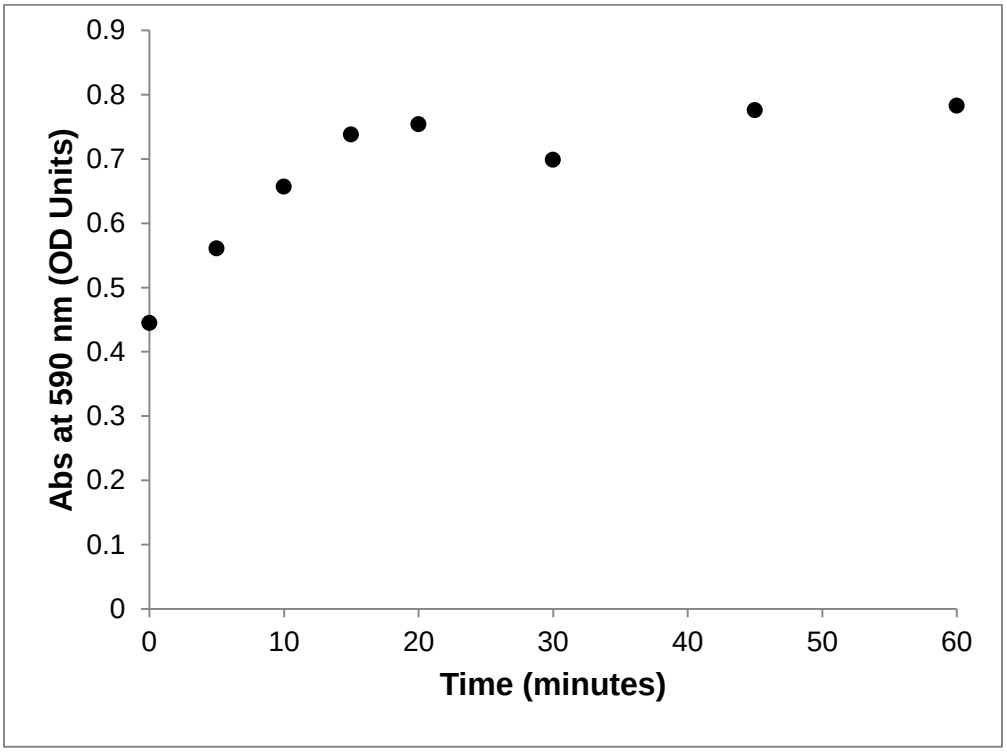

FIGURE 4. **Kinetic analysis of EPS degradation.** Depolymerase protein (final concentration: 3.6 ng μL^{-1}) was incubated with EPS (0.5 µg μL^{-1}) in 150 mM NaCl, 25 mM Tris pH 7.2 at 37 °C. As before, the concentration of reducing ends in each aliquot was measured by the BCA assay.

Metal dependence of the reaction

To determine if the depolymerase depends on divalent metal cations for its function, the kinetic analysis was repeated with the chelating agent EDTA. Figure 5 shows the initial velocity of activity of the enzyme is reduced to 59% of its original value when incubated with 5 mM EDTA.

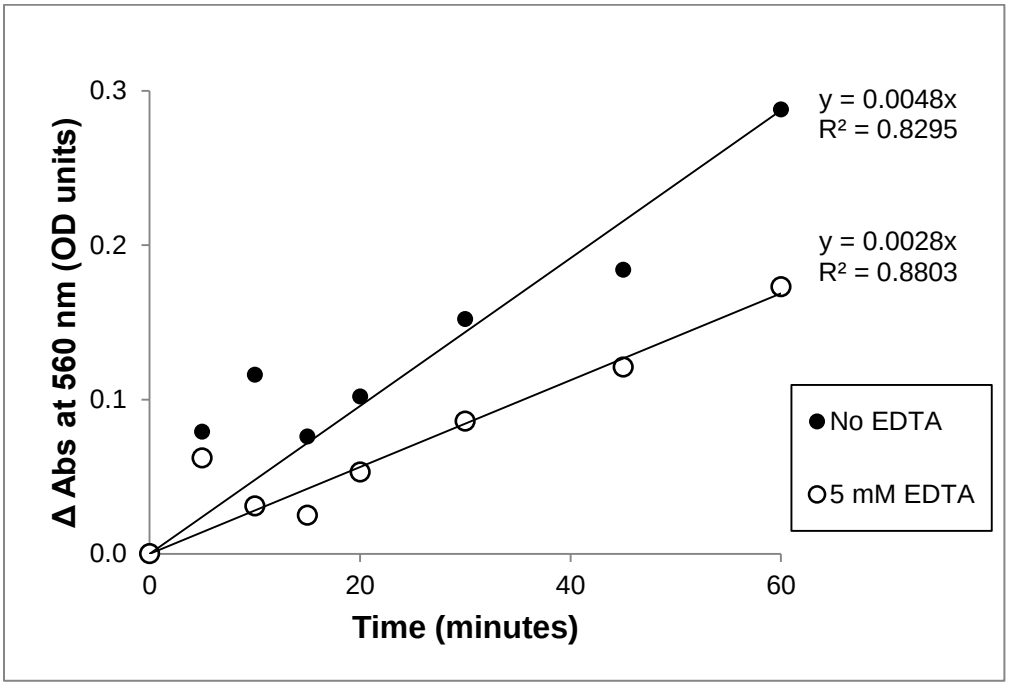

FIGURE 5. **Divalent cation dependence of the depolymerase.** Protein (final concentration: 5.6 ng μL^{-1}) was incubated with EPS: (0.5 µg µL-1) in 150 mM NaCl, 25 mM Tris pH 7.2 at 37 °C. EDTA was added to one reaction (open circles) to a final concentration of 5 mM.

Incubation time affects *A. baumannii* EPS composition

Purification of the EPS was performed twice. Plates containing *A. baumannii* were incubated for either 72 h or 120 h. To determine the reducing end content of the undigested sugar, standard curves were constructed against glucose. Figure 6 shows the 120 h EPS contains half the number of reducing ends per unit mass compared with the 72 h EPS. The experiment was repeated once with similar results.

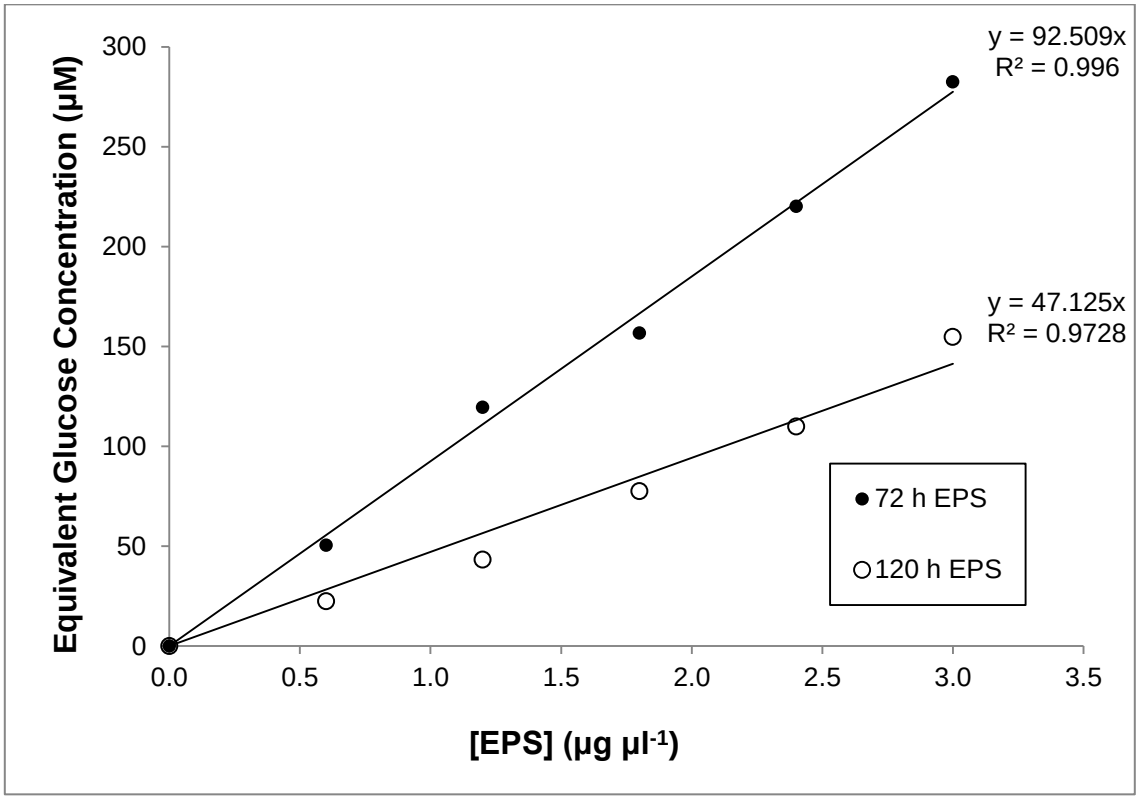

FIGURE 6. **Standard curves of EPS isolated at 72 h and 120 h.** Six independently prepared EPS samples of varying concentrations were used to establish standard curves. Absorbances were converted into equivalent glucose concentrations by establishing a glucose standard curve (data not shown). “Equivalent glucose concentration” is defined as the concentration of glucose required to achieve an absorbance in the BCA assay equivalent to that of a particular sample of EPS.

Degradation of 72 h vs. 120 h EPS

To determine the reducing end content of the digested sugar, both EPS samples were incubated under identical conditions with the depolymerase for 2 h. Figure 7 shows the depolymerase degrades the 120 h EPS into 181 μM glucose equivalent reducing ends, compared to 162 μM for the 72 h EPS.

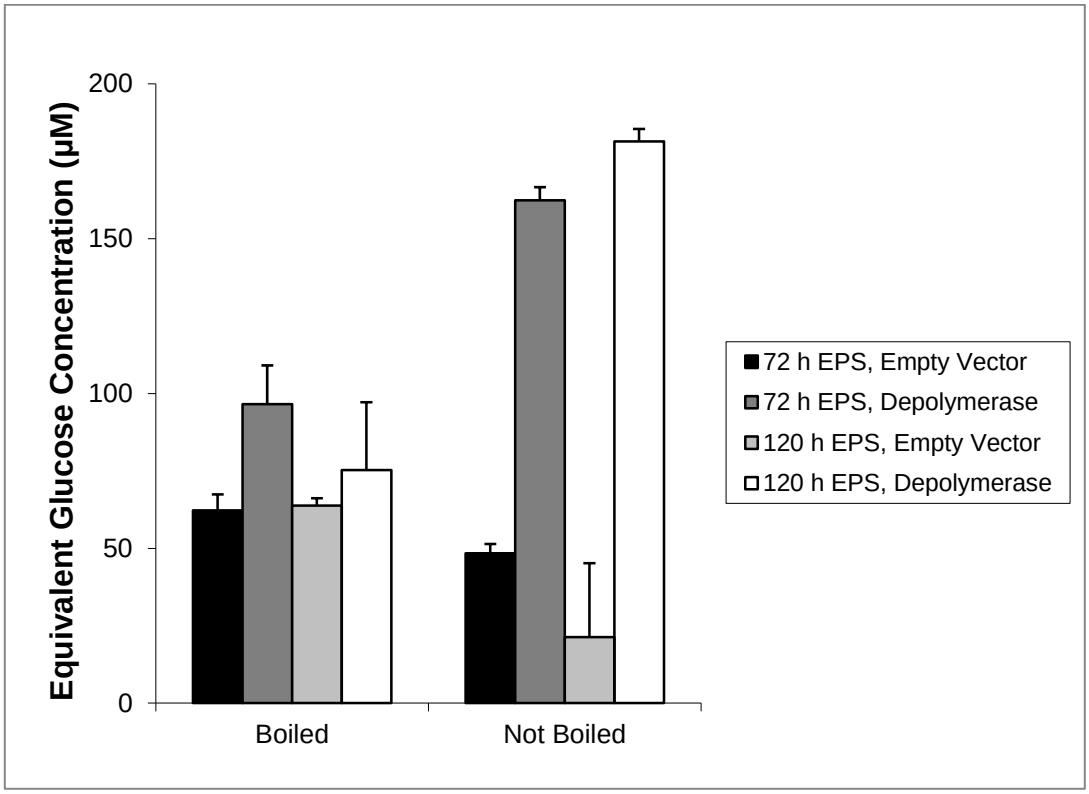

FIGURE 7. **Degradation assay with 72 h and 120 h EPS.** The assay was carried out as in Figure 2, except that the reactions were incubated for 2 h (*n*=5 reactions). "Empty vector" refers to a strain carrying the pET28B vector with no insert, induced, and the lysate subjected to purification just as that of the vector carrying the depolymerase. Error bars represent standard error.

The depolymerase forms multimers in solution

The multimeric state of the protein was analyzed by size exclusion chromatography (Figure 8). Calibration of the column with protein size standards yielded the equation $M_r = exp(-7.69 * K_{av} + 14.28)$, where M_r is the apparent molecular mass based on migration through the column. The protein migrated in a single peak at an elution volume of 10.0 – 12.0 mL, which corresponds to $M_r = 637$ kDa. A second late-eluting peak (elution volume: 17.0 – 18.5 mL) did not contain any of the depolymerase protein. The molecular mass of the monomer is 102.4 kDa, approximately 1/6th of its apparent mass on the size-exclusion column.

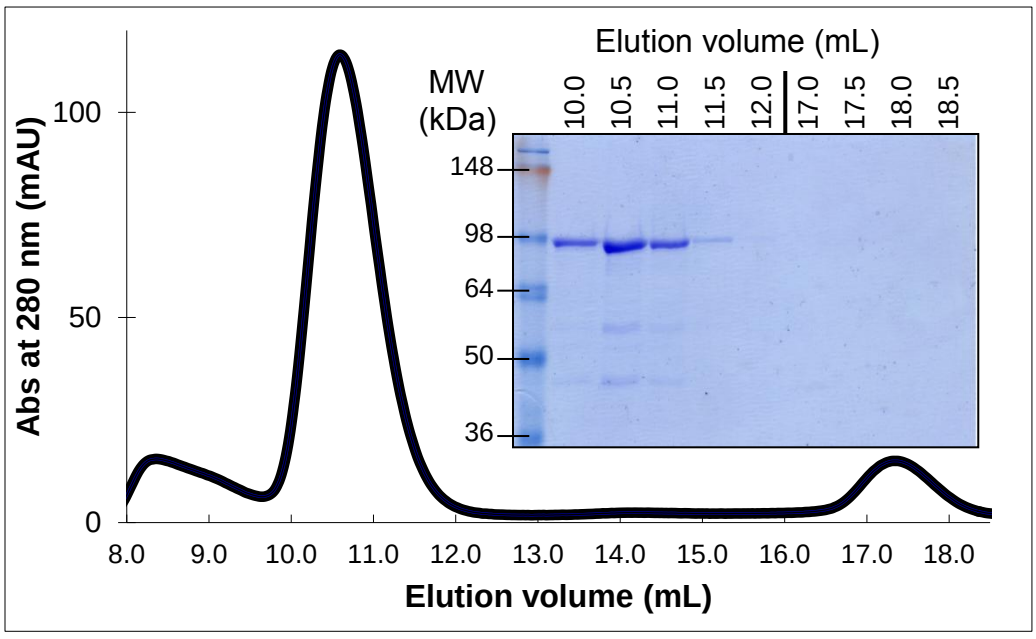

FIGURE 8. **Oligomerization state analysis by size exclusion chromatography.** The elution profile of the depolymerase protein on an S200 size exclusion column. Inset: the 0.5 mL fractions were analyzed on a Coomassie-stained SDS-PAGE gel.

The depolymerase degrades *A. baumannii* biofilms

To test the ability of the depolymerase to disperse *A. baumannii* biofilms, biofilms were grown on a 96-well polystyrene plate and depolymerase protein was incubated with the biofilms for 10 h. Figure 9 shows the remaining biofilm from two *A. baumannii* strains after depolymerase treatment as compared with untreated controls. The depolymerase removed 24% of the biofilm from strain AU 0783 and 74% of the biofilm from strain Iraq 1887.

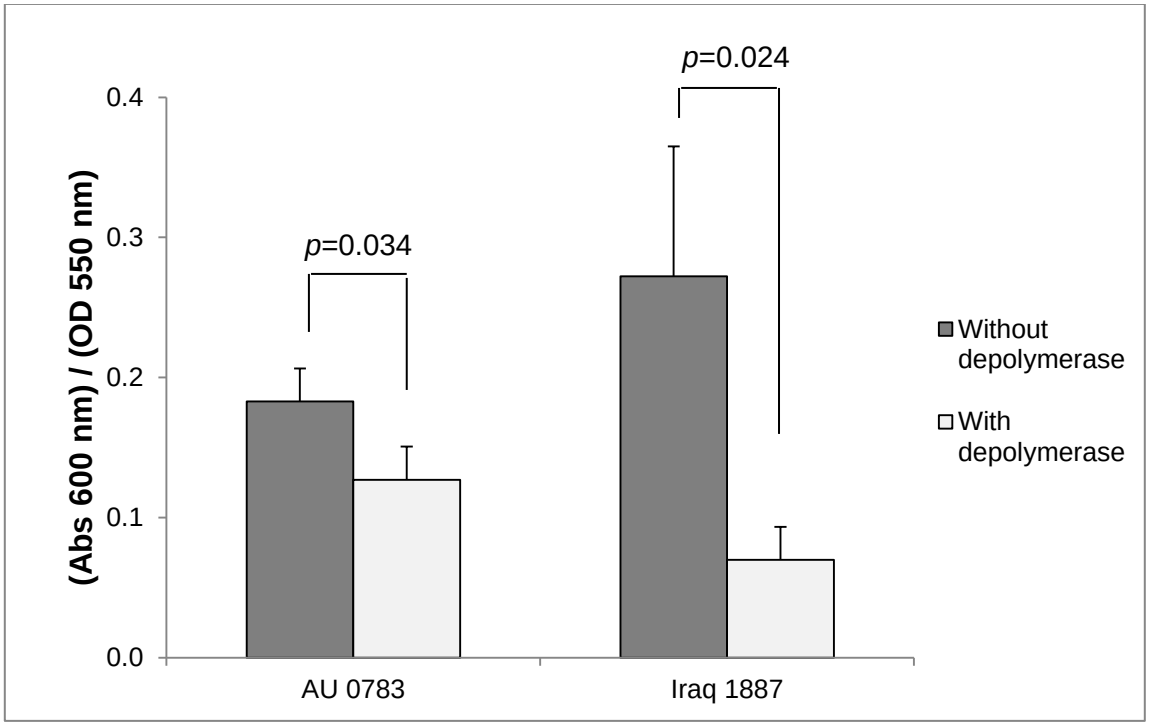

FIGURE 9. **Removal of *A. baumannii* biofilms**. The amount of biofilm in each well was quantified by measuring the absorbance of CV dye in the well (A_{600}) and normalizing to the cell density (OD_{550}) in that well before depolymerase treatment (Y axis). Bar height indicates the mean of eight wells for each condition (*n*=8). Probabilities are for a one-tailed student's T-test. Error bars represent standard error.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Bacteriophages represent a new source of tools to use against bacterial infections. The experiments in this study demonstrate that a novel phage-derived protein, AbauYa1 gp38, degrades *A. baumannii* capsular polysaccharide and biofilms, two major contributors to *A. baumannii* virulence.

Protein purification

Efficient protein purification is essential to biochemical studies of any protein. Figure 1 shows that half of the protein was lost in the pellet. Although yields are already quite high, they could be increased even further by recovering protein from the pellet. Phage tail spikes are prone to forming insoluble aggregates, and some labs have devised protocols to recover protein stuck in the pellet (14). Optimizing this step will greatly improve yields.

EPS structure and yield

A. baumannii secretes extracellular polysaccharide (EPS) that is crucial for biofilm formation and virulence (5). The EPS changes composition over time, as evidenced by drastically altered yields in EPS purification (Table 3) and differences in reducing end content (Figure 6). Even though undigested EPS isolated at 72 h contains twice the amount of reducing ends as EPS isolated at 120 h (Figure 6), fully digested 72 h EPS generates 90% of the reducing ends as 120 h EPS (Figure 7). The most parsimonious

explanation is that the EPS is composed of identical repeating units, and the bacterium increases the chain length of the polysaccharide over time by incorporating additional repeating units.

The structure of the EPS is unknown. Parameters such as k_{cat} and K_m cannot be determined for the depolymerase without a chemically defined substrate and products. Determination of the structure of the EPS and regulation of EPS secretion in *A. baumannii* are promising lines of research that will give insights into depolymerase enzymology and *A. baumannii* pathogenesis, respectively.

Polysaccharide depolymerase activity

A recent study showed that genes involved in the biosynthesis and export of extracellular polysaccharide are essential for *A. baumannii* pathogenesis in a rat soft tissue infection model (5). Since *A. baumannii* EPS is a virulence factor, degrading the EPS is a plausible therapeutic strategy for treating *A. baumannii* infections. The results shown in Figures 3 and 7 demonstrate that AbauYa1 gp38 possesses polysaccharide depolymerase activity. The assays with boiled protein demonstrate that the EPS does not spontaneously hydrolyze under the assay conditions, and the assays with an empty vector demonstrate that no residual *E. coli* proteins are responsible for this activity. The increase in reducing ends comes from cleavage of the bonds linking the full-length EPS and only occurs when active protein is incubated with EPS.

Oligomeric structure

Several phage tail fibers are known to form trimers in solution, including those from phage P22 (15) and *E. coli* K1 phages (16). The observation that the depolymerase migrates as approximately hexamers on a size exclusion column is consistent with the fact that other phage tail fibers form multimeric complexes, but no other tail fiber is known to form a hexamer. Further analysis, such as running the protein on an SDS-PAGE gel without boiling or analytical ultracentrifugation, will provide additional data that will elucidate the true multimeric state of the depolymerase.

Biofilm degradation

The depolymerase removes *A. baumannii* biofilms adherent to polystyrene surfaces. Crystal violet dye stains both the cells remaining in the biofilm and the extracellular components of the biofilm, so it is an accurate readout of the amount of biofilm attached to a surface (13). Figure 9 shows the decrease in CV retained, and hence adherent biofilm, upon incubation with the depolymerase. However, several issues must be resolved before medical applications of the depolymerase become possible. First, biofilm removal is incomplete. *A. baumannii* may remain attached to the surface because it secretes proteins that mediate direct attachment to environmental surfaces (17), or the depolymerase may be physically blocked from accessing EPS deep within the biofilm under the timeframe of the experiment. Second, the effect is strain-dependent. The chemical structure of the *A. baumannii* capsule as well as the structural determinants of polysaccharide depolymerase specificity are completely unknown. Elucidation of the

structural basis of depolymerase activity represents a valuable line of research toward being able to rationally engineer depolymerases. Last, the results are from an *in vitro* experiment and remove *A. baumannii* biofilms from a polystyrene surface. The depolymerase may not be as effective in removing biofilms *in vivo* from a wound surface. Despite these caveats, the depolymerase certainly has valuable therapeutic potential as a tool to treat *A. baumannii* biofilms.

Conclusions

AbauYa1 gp38 is a virion-associated polysaccharide depolymerase that degrades the exopolysaccharide of *A. baumannii*. Additionally, the depolymerase partially removes *A. baumannii* biofilms adherent to a polystyrene surface. Thus, the depolymerase is of high therapeutic potential for treating *A. baumannii* infections.

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