Structure and Lytic Activity of a Bacillus anthracis Prophage Endolysin

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Lih Yoon Low,*†,*,†,‡,‡1 Chen Yang,*†,‡,‡1 Marta Perego,*‡, Andrei Osterman,*‡ and Robert C. Liddington*‡‡

From the †Infectious and Inflammatory Disease Center, The Burnham Institute and the ‡Division of Cellular Biology, Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, California 92037

We report a structural and functional analysis of the β-prophage Ba02 endolysin (PlyL) encoded by the Bacillus anthracis genome. We show that PlyL comprises two autonomously folded domains, an N-terminal catalytic domain and a C-terminal cell wall-binding domain. We determined the crystal structure of the catalytic domain; its three-dimensional fold is related to that of the cell wall amidase, T7 lysozyme, and contains a conserved zinc coordination site and other components of the catalytic machinery. We demonstrate that PlyL is an N-acetylmuramoyl-L-alanine amidase that cleaves the cell wall of several Bacillus species when applied exogenously. We show, unexpectedly, that the catalytic domain of PlyL cleaves more efficiently than the full-length protein, except in the case of Bacillus cereus, and using GFP-tagged cell wall-binding domain, we detected strong binding of the cell wall-binding domain to B. cereus but not to other species tested. We further show that a related endolysin (Ply21) from the B. cereus phage, TP21, shows a similar pattern of behavior. To explain these data, and the species specificity of PlyL, we propose that the C-terminal domain inhibits the activity of the catalytic domain through intramolecular interactions that are relieved upon binding of the C-terminal domain to the cell wall. Furthermore, our data show that (when applied exogenously) targeting of the enzyme to the cell wall is not a prerequisite of its lytic activity, which is inherently high. These results may have broad implications for the design of endolysins as therapeutic agents.

Endolysins are bacteriophage-encoded enzymes that lyse the host bacterial cell wall during the lytic phase of the phage infectious cycle. They typically consist of an N-terminal catalytic domain and a C-terminal domain that targets the enzyme to the cell wall, providing high specificity and strain specificity (1, 2). For example, the Listeria monocytogenes lysins, Ply118 and Ply500, specifically hydrolyze Listeria cells but are inactive in the absence of the cell wall-binding domain (1).

A comparative genome analysis of Bacillus anthracis revealed a gene encoding a putative endolysin within the integrated copy of the β Ba02 prophage, which we will call PlyL. PlyL has a high degree of sequence similarity in its catalytic domain with an endolysin from the bacteriophage γ PlyG (3, 4), which specifically lyases and kills B. anthracis and closely related species when added exogenously to bacterial cultures. For this reason, PlyG is being developed as a diagnostic and therapeutic agent (5).

Here we describe a structural and functional analysis of PlyL. We show that the N-terminal (catalytic) domain is an amidase with high inherent lytic activity against the cell wall of several Bacillus species. In contrast to many previously described lysins, we find that the C-terminal domain plays a dual role, not only as a cell wall targeting domain but also as an inhibitor of catalytic activity in the absence of the cognate target.

MATERIALS AND METHODS

Cloning and Expression of Full-length Endolysin and C-terminal Domain—Full-length PlyL was cloned by PCR from the Bacillus anthracis Ames strain total DNA extract provided by Dr Phil Hanna (University of Michigan Medical School) using the oligonucleotide primers 5′-AAAGGAGATATACATATGGAAATCAGAAAAATTAGTT-3′ (forward) and 5′-GAATTGGATCTCATTTATTAGTTCATACACAATC-3′ (reverse). We used the forward primer 5′-GGAGATATACATATGGCAAGTGCAACGGTAACCCCTAATAA-3′ with the same reverse primer. PCR products were cloned into pET22b (Novagen) via NdeI and BamHI restriction sites (without tag). The resulting plasmids were transformed into BL21DE3 (Novagen) for protein expression. All protein constructs were expressed using the same protocol. Transformed cells from overnight plates were used to inoculate 1 liter of 2X TY medium (16 g/liter Tryptone, 10 g/liter yeast extract, and 5 g/NaCl supplemented with 100 μg/ml ampicillin), and allowed to grow to A600 of 1.0 at 37 °C. 1 mM isopropyl 1-thio-β-D-galactopyranoside was added to induce protein expression over 3 h at 37 °C.

Full-length PlyL Purification—Cells were harvested by centrifugation at 4 °C. 30 ml of lysis buffer (50 mM Na-Mes, pH 6.0, 10 mM β-mercaptoethanol, 0.1% Triton X-100, and 0.1 mM ZnSO4) was used to resuspend the cell pellet. Resuspended cells were lysed by sonication and clarified by centrifugation for 1 h at 4 °C. Clarified lysate was loaded directly into a HITRAP 5 ml SP column on an Akta FPLC (Amersham Biosciences) equilibrated with 50 ml of buffer A (50 mM Na-Mes, pH 6.0, 10 mM β-mercaptoethanol, and 0.1 mM ZnSO4). Unbound protein was eluted with the column with 50 ml of buffer B. A gradient of 0–1 M NaCl in buffer A with a total volume of 50 ml was applied to the column to elute the protein. Fractions containing the full-length PlyL, more than 90% pure as verified by SDS-PAGE, were pooled and concentrated to 10–20 mg/ml. A final gel filtration column, Superdex 75 (16/60; Amersham Biosciences), was then used to further purify the protein.

Cloning, Expression, and Purification of Ply21 Constructs—The gene encoding Ply21 was provided by Dr. Martin Loessner (Inst.f.Lebensmit-

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1 Both authors contributed equally to this work.

2 To whom correspondence should be addressed: The Burnham Institute, 10901 North Torrey Pines Rd., La Jolla, CA 92037. Tel.: 858-646-3136; Fax: 858-713-9925; E-mail: rlidding@burnham.org.

The abbreviations used are: Mes, 4-morpholineethanesulfonic acid; GFP, green fluorescent protein; CBD, cell wall-binding domain.
Bacillus Prophage Endolysin Structure

tel-ü.Ernährungswissens ETH-Zentrum Zürich). An internal NdeI was silence-mutated using an overlap-extension PCR technique. Full-length Ply21 (263 amino acids) and its N-terminal domain (amino acids 1–159) were subcloned into pET15b via NdeI and BamHI sites. Bacterial cell extracts were prepared as described above. The supernatant was loaded onto the equilibrated nickel-nitrilotriacetic acid column and washed with 10 column-volumes of wash buffer (50 mM Tris-Cl, 300 mM NaCl and 30 mM imidazole at pH 7.5). The elution buffer was similar to the wash buffer but included 300 mM imidazole. Thrombin was used to remove the N-terminal His-tag at room temperature for at least 24 h. The cleaved proteins was then purified by Superdex 75 (16/60).

Purification and Crystallization of the PlyL Catalytic Domain—The N-terminal catalytic domain was generated by limited proteolysis of the full-length PlyL using elastase at a ratio of 1:100 at room temperature for 16 h. A Superdex 75 16/60 column (Amersham Biosciences) was used as a final column to purify the catalytic domain. The buffer was 20 mM Tris-Cl, pH 7.0, 100 mM NaCl, 10 mM β-mercaptoethanol. The final purified protein was concentrated to 20 mg/ml. Mass spectrometry and amino acid analysis revealed that elastase cleaved after residue Val-159. The protein appeared as a single band on SDS-PAGE, and the molecular weight was confirmed by matrix-assisted laser desorption ionization-mass spectrometry. Crystals were obtained by hanging drop vapor diffusion at 20 °C, using a reservoir of 0.6M NaH2PO4, 1.0M K2HPO4, 0.1M acetate at pH 6.7. Each drop consisted of 2 μl of protein and 1 μl of buffer. Crystals grew as hexagonal rods to 0.1 mm × 0.1 mm × 0.3 mm in 3 days at room temperature. They adopt space group P61 with cell dimensions a = 90.7 Å, b = 90.7 Å, c = 373 Å. To prepare for cryo-x-ray data collection, the crystals were soaked in a series of steps with crystallization buffer containing glycerol to a final concentration of 20%. All x-ray data sets were collected at 100 K.

PlyL C-terminal Domain Purification and Crystallization—The purification protocol was identical to that of the His-tagged Ply21 constructs described above. Crystals of the C-terminal 75 amino acid domain were obtained by equilibration against 1.5 M (NH4)2SO4 and 10% glycerol in Tris-Cl, pH 7.0, by hanging drop vapor diffusion. The crystal grew to a size of 0.1 × 0.1 × 0.3 mm3 in 7 days at room temperature; they diffract to 2.7 Å resolution using a Rigaku FR-E High Brillance X-Ray generator and adopt space group P41212 with cell dimensions a = 224.2 Å, b = 52.5 Å, c = 224.2 Å.

Structure Determination of the PlyL Catalytic Domain—Multiwavelength anomalous diffraction data sets were collected at beamline 9-2 at the Stanford Synchrotron Radiation Laboratory using a MAR345 image plate and processed using the programs DENZO and SCALEPACK (6). The presence of a zinc ion in the crystal was confirmed by a fluorescence scan at the zinc L-1 edge. 18 selenomethionine sites were found using SOLVE (7) and used for phase calculation to a resolution of 2.0 Å. An initial model was generated by RESOLVE (8), further model building was done using O (9), and the model was refined with CNS (10) (version 1.1 on Mac OS X). Native crystals were obtained under identical conditions. A data set was collected in-house with a Rigaku FR-E High Brillance X-Ray generator using the R-axis IV detector. The CNS-refined model of the selenomethionine structure was used as the input template for native refinement to a resolution of 1.86 Å. There are three molecules (A–C) in the asymmetric unit with essentially identical structure (root mean square deviations on Ca coordinates of 0.29 Å) and a solvent content of 46%. Density for the last two amino acids in the molecules A and C are missing. Molecule B has the most complete density throughout, and its B-factors are lower than for the other two molecules. Refinement statistics are presented in TABLE ONE. The coordinates and structure factors have been deposited with the PDB with accession code 3YB0. The catalytically inactive mutant E90A crystallized isomorphously with the wild-type and showed only small differences in the vicinity of the mutation site.

Assay of Lytic Activity—The activity of PlyL and Ply21 when applied exogenously to cultures of B. anthracis (Sterne 34F2), Bacillus cereus ATCC 4342, Bacillus megaterium WH320, Bacillus subtilis 168 and Escherichia coli CFT073 were tested. Cultures were grown to mid-exponential phase, and cells were harvested and resuspended in 10 mM sodium phosphate, pH 7.0. The lysis of cell suspensions upon addition of 2–4 μl pure endolysin samples was monitored at 600 nm.

 Determination of the Cleavage Site in Peptidoglycan—Peptidoglycan suspension (0.5 mg/ml) from B. subtilis (Fluka) was incubated at 37 °C with purified PlyL (0.4 μl) in 10 ml of Good’s buffer (20 mM Na-MES, pH 6.5) containing 100 mM KCl. Boiled PlyL was used as a control. After incubation for 30, 60, and 120 min, samples were boiled and centrifuged at 13,000 rpm/min, clear supernatants were analyzed for the release of free amino acids using a modified protocol described in Ref. 12. 100-μl aliquots were mixed with 12 μl of 10% K2B4O7, and 10 μl of 1-fluoro-2,4-dinitrobenzene solution (0.1 μl in ethanol) was added, and the mixture was heated at 65 °C for 45 min in the dark. Following acid hydrolysis in 4 M HCl for 12 h at 95 °C, the 2,4-dinitrophenyl-labeled compounds were analyzed by HPLC on a reverse-phase column (C18, 4.6 × 150 mm, Vydac). The labeled amino acids were eluted with a linear gradient from 90% A + 10% B to 30% A + 70% B (A, 10% acetonitrile in 20 mM acetic acid; B, 90% acetonitrile in 20 mM acetic acid), and detected at 365 nm. The release of free reducing groups during the enzymatic reaction was measured by a modified Morgan-Elson reaction (12) using N-acetylgalactosamine as the standard.

C-terminal Domain Cell Binding Assay—A modified green fluorescent protein (GFP) gene (gift of Dr Ruchika Gupta) was PCR-amplified using the following oligonucleotide primers: 5’-CGGCGGCCGC- CATATGTTGAGCAAGGGCGAGGAGCTGTTC-3’ and 5’-GC CCGGATCTCGAGTTACTTGTACAGCTGGTCCATGCCC-3’. The resulting fragment was digested by NdeI and XhoI (underlined) and ligated with the XhoI-BamHI fragment of the C-terminal domain of PlyL, which was amplified using 5’-AGCCATATGCTCGAGATGGCAAGTCGACGATGGCCAATGCACCGTAGATGAGAGCCATACCTCA-3’ (forward) and the same reverse oligonucleotide that was used for the cloning of the full-length protein. The GFP-C-terminal domain fusion and a GFP control were cloned into a pET15b vector via NdeI and BamHI or XhoI, respectively. Both proteins were expressed and purified using nickel-nitrilotriacetic acid affinity chromatography and gel-filtration as described above. Cell samples for the binding assays were obtained by growing Bacilli cultures to late log phase. Cells were harvested, washed with PBS-T (phosphate-buffered saline + 0.1% Tween 20), and incubated with 0.4 μM protein samples (GFP-C-domain fusion or GFP control) for 5 min at room temperature, prior to three washes with PBS-T. The washed cells were smeared onto a microscope slide for confocal image analysis with the Bio-Rad Radiance 2100 Multiphoton Laser Scanning Confocal Microscope system equipped with Argon laser (Image Analysis and Histology Facilities, The Burnham Institute). The objective used was 60X LSM with oil immersion and zoom 5 on the N.A.1.0 (Olympus) microscope. The wavelength of 488 nm was used to excite the GFP.

RESULTS

Identification and Characterization of PlyL—A Blast search (http://ncbi.nlm.nih.gov/BLAST/) using the γ phase endolysin, Plyγ, as the query sequence identified two genes encoding putative endolysins located within an integrated prophage of B. anthracis. The A Ba01 and A Ba02 endolysins are annotated as BA3767 and BA4073 (“PlyL”), respec-
tively, in the genome sequence of *B. anthracis* Ames (NCBI accession number NC_003997). Additional endolysins from other *Bacillus* species and their phages were also detected in this search. Those with greater than 30% identity over their catalytic domains are shown in Fig. 1. PlyL is most closely related to PlyG in both the enzymatic (93% identity) and C-terminal (60% identity) domains. BA3767 is also very similar but lacks the C-terminal domain.

We cloned and expressed a *B. anthracis* gene encoding BA4073/PlyL. Crystallization trials of the full-length protein were unsuccessful. However, limited proteolysis using elastase allowed us to isolate a stable N-terminal fragment (residues 1–159). Cleavage occurs at the junction between the predicted catalytic and cell wall-binding domains. This fragment was much more soluble than the full-length protein (40 mg/ml versus 3 mg/ml), and crystallized readily. We also crystallized the C-terminal domain; although we have not yet solved its structure, the existence of crystals that diffract to high resolution indicates that it is an autonomously folded domain.

**N-Acetylmuramoyl-L-alanine Amidase Activity of PlyL Resides in Its N-terminal Domain**—To assess the enzymatic activity of PlyL, peptidoglycan from *B. subtilis* was treated with full-length PlyL and the elastase-generated N-terminal fragment. No increase in free reducing groups derived from peptidoglycan could be observed, indicating that the enzyme is neither a glucosaminidase nor a muramidase. The free amino groups of the digested (solubilized) products were labeled with 1-fluoro-2,4-dinitrobenzene. After acid hydrolysis, the 2,4-dinitrophenyl-labeled compounds were separated by high pressure liquid chromatography. Only the amount of 2,4-dinitrophenyl-alanine was increased significantly (supplemental Fig. 1A), which indicates that the enzyme is an *N*-acetylmuramoyl-L-alanine amidase, specifically cleaving the amide bond between *N*-acetylmuramic acid and L-alanine. The same result was observed for the N-terminal proteolytic fragment, showing that it comprises a complete catalytic domain. The N-terminal domain was more active than the full-length protein in this assay (supplemental Fig. 1B), providing the first indication that the C-terminal domain is autoinhibitory.

**Structure of the PlyL N-terminal Domain**—We solved the structure of the PlyL catalytic domain (residues 1–159) at 1.86 Å resolution using multiwavelength anomalous diffraction phasing from a selenomethionine-substituted protein (TABLE ONE). The fold is most similar to those of the T7 lysozyme (13), *Citrobacter* AmpD (14), and the *Drosophila* peptidoglycan recognition protein PGRP-LB (15), with which it shares 10–20% identity. For consistency, we have followed the strand and helix nomenclature of T7 lysozyme. The overall fold consists of a six-stranded β-sheet flanked by four long β-helices (one at the front (β1) and three at the back (α2, α3, and α4) as well as a number of elaborate loops with short α-helical segments (Figs. 2 and 3A). Compared with T7 lysozyme, an N-terminal extension creates an additional β-strand (β0) at one end of the sheet. A zinc ion binds to the front face of the molecule at the center of the active site, coordinated by His-29 from strand α1 and three at the back (α2, α3, and α4) as well as a number of elaborate loops with short α-helical segments (Figs. 2 and 3A). Compared with T7 lysozyme, an N-terminal extension creates an additional β-strand (β0) at one end of the sheet. A zinc ion binds to the front face of the molecule at the center of the active site, coordinated by His-29 from strand β1, and by two residues, His-129 and Cys-137, on either side of strand β3. The fourth ligand is a phosphate (or sulfate) ion from the crystallization buffer.

**Active Site**—The active site is solvent-exposed and lies in a shallow groove on the protein surface, consistent with the ability to cleave a
highly cross-linked and branched polymer. Helix α1 packs more closely against the β-sheet in PlyL than in T7 lysozyme, so that the pronounced substrate-binding groove observed for T7 lysozyme is not seen for PlyL. The active site can be overlaid closely with that of T7 lysozyme (Fig. 3B). The three zinc-coordinating residues (His-29, His-129, and Cys-137) are conserved between PlyL and T7 lysozyme (the third zinc-coordinating residue is an Asp in Citrobacter AmpD). PlyL Lys-135 is structurally analogous to Lys-128 of T7 lysozyme, which has been shown to be important for catalysis (13), perhaps by stabilizing the developing negative charge on the amide carbonyl in the transition state; however, PGRP-LB has a threonine at this position. Tyr-46 in T7 lysozyme and Tyr-78 in PGRP-LB are important for catalysis and are thought to act as the general base to activate the nucleophilic water molecule. On the basis of sequence alignment the analogous residue in PlyL was predicted to be Phe-53. However, in the crystal structure the side chain of Phe-53 adopts a different orientation and the carboxylate group of Glu-90 (from a neighboring strand) occupies the space analogous to the T7 tyrosine. To demonstrate a catalytic role for Glu-90 in PlyL, we mutated it to alanine, and indeed this mutation completely abolished the amidase activity. The mutant is correctly folded as judged by its ability to crystallize isomorphously with the wild-type protein (data not shown).

There are only 10 amino acid residues different within the N-terminal domains of PlyL and PlyG, so that their three-dimensional structures should be almost identical. These differences are plotted on the three-dimensional model of PlyL (Fig. 3A). Most of the differences are located on the surface of the molecule, and all of them are distant from the active site and a putative substrate binding cleft, suggesting that the two catalytic domains should have similar or identical substrate specificity and catalytic activity.

Lytic Activity of PlyL and Ply21—We next examined the lytic activity of PlyL on whole cells of several bacilli, as measured by light scattering. We found, unexpectedly, that the N-terminal catalytic domain of PlyL is more active than the full-length protein in lysing B. subtilis and some strains of B. anthracis. However, in marked contrast with PlyG, a relatively high lytic activity of PlyL was established on B. megaterium and lower but detectable activity on B. subtilis and B. anthracis.

We found, unexpectedly, that the N-terminal catalytic domain of PlyL is more active than the full-length protein in lysing B. cereus with an efficiency comparable with that reported for PlyG on B. anthracis and some strains of B. cereus (5). However, in marked contrast with PlyG, a relatively high lytic activity of PlyL was established on B. megaterium and lower but detectable activity on B. subtilis and B. anthracis.
FIGURE 2. Three-dimensional structure of PlyL catalytic domain and related amidases. Molscript (version 2.1 (21, 22)) ribbon representations of the structures of PlyL, T7 lysozyme (PDB: 1LBA), PGRP-LB (PDB: 1OHT), and AmpD (PDB: 1J3G). The zinc ion is shown as a gray sphere. The colors represent the secondary structure arrangement. The backbone RMS differences with T7 lysozyme and PGRP-LB, are 1.8 Å (for 107 atoms) and 2.0 Å (for 106 atoms), respectively. The N and C termini are labeled N and C. The N termini of PlyL, PGRP-LB, and AmpD are at the back of the β-sheet.

FIGURE 3. Stereo views of PlyL and active site comparisons. A, stereo Co representation of PlyL. Amino acids differences between PlyL and PlyG are indicated. Most of these are surface-exposed except for Val-55, which makes hydrophobic contacts with Trp-68 in PlyL. In PlyG, the Val-55 is replaced by the larger residue Ile, but this is complemented by a change to the smaller Leu in place of Trp-68. B, stereo view of the active site residues of PlyL (light gray), T7 lysozyme (PDB: 1LBA) (medium gray), and PGRP-LB (PDB: 1OHT) (dark gray).
domain has no obvious homology. Ply21 has previously been shown to lyse *B. cereus* strains when added exogenously, whereas *B. subtilis* cells are resistant (16). We confirmed this specificity for the full-length endolysin, but found a dramatic increase in lytic activity toward *B. subtilis* by the free N-terminal domain. In contrast, lysis of *B. cereus* by the N-terminal domain was significantly reduced compared with the full-length enzyme (Fig. 4D).

To further assess the role of the C-terminal domain of PlyL, we performed cell binding studies using a recombinant C-terminal domain fused with GFP. When added to *B. cereus* and viewed under a confocal microscope, a clear green fluorescence can be observed around the cells (Fig. 4E). No binding was observed with *B. megaterium* or *B. subtilis* (*B. anthracis* was not tested).

**DISCUSSION**

We have shown that the endolysin from the *B. anthracis* λ prophage Ba02, PlyL, is a *bona fide* cell wall lytic amidase with a modular organization comprising an N-terminal catalytic domain and a C-terminal cell wall binding domain.
The constraints on the catalytic domain. Similar results were found for
we propose that strong binding of the CBD to the target cell wall releases
sensitive to the amidase activity. In the case of
difference in the activity of the full-length PlyL and the free N-terminal
domain thus relieving the inhibitory effect. For example, the marked
which disrupts the interaction between the CBD and the catalytic
domain participates in species-specific cell wall binding (recognition),
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We therefore propose the following model (Fig. 5); the CBDs of PlyL
and Ply21, because we observed a very similar pattern of activity for a phage
endolysin that is specific for B. cereus, Ply21. This protein has the same
domain organization as that of the PlyL, and a similar catalytic domain
but has little sequence similarity in the C-terminal cell wall-binding
domain. It appears that in both cases the cell wall-binding domain
lies close to the active site, so that the distinct lytic specificities of the
full-length proteins are presumably endowed by the C-terminal
domain, which is less well conserved.

We showed that the C-terminal domain is indeed a cell wall-binding
domain (CBD) and that it interacts specifically with B. cereus cells. We
further showed that the presence of the CBD within the full-length PlyL
has an inhibitory effect on the lytic activity of the catalytic domain when
tested with peptidoglycan or with the whole cells of B. subtilis and, to a
lesser extent, with B. megaterium and B. anthracis. By contrast, the
presence of the CBD had a barely significant effect on the activity of PlyL
toward B. cereus. We established that this behavior is not a peculiarity of
PlyL, because we observed a very similar pattern of activity for a phage
endolysin that is specific for B. cereus, Ply21. This protein has the same
domain organization as that of the PlyL, and a similar catalytic domain
but has little sequence similarity in the C-terminal cell wall-binding
domain. It appears that in both cases the cell wall-binding domain
serves as an additional level of selectivity by negatively regulating the
catalytic domain and only allows the catalytic domain to function effect-
ively in the presence of a specific cell wall.

We therefore propose the following model (Fig. 5); the CBDs of PlyL
and Ply21 have dual functions. (i) In the absence of specific interaction
with cognate cell wall, the CBD plays an autoinhibitory role, similar to a
propeptide in zymogens. Given the structure of the catalytic domain, it
is likely that the inhibition is allosteric, because the C terminus of the
domain protrudes from a surface that is distal to the active site. (ii) The
CBD participates in species-specific cell wall binding (recognition),
which disrupts the interaction between the CBD and the catalytic
domain thus relieving the inhibitory effect. For example, the marked
difference in the activity of the full-length PlyL and the free N-terminal
domain against B. subtilis can be explained by very weak binding of the
CBD to the B. subtilis cell wall, whereas the cell wall is intrinsically
sensitive to the amidase activity. In the case of B. cereus where the
full-length and truncated enzymes have an almost equally high activity,
we propose that strong binding of the CBD to the target cell wall releases
the constraints on the catalytic domain. Similar results were found for
Ply21. In that case, localization of the enzymatic domain to the cell
surface significantly enhances the rate of lysis, presumably via a local
concentration effect.

Endolysins are generally observed to be highly specific toward a par-
ticular species of bacteria, by virtue of their distinct CBDs that recognize
variable cell wall structures (1, 2). Our observation that the catalytic
domain of PlyL has strong lytic activity against a number of different
Bacillus species and that this activity does not require (or is inhibited by)
the CBD suggests either that the PlyL family of endolysins are atypical or
that the kinetics of lysis are different when the lysin is applied exog-
nenously rather than endogenously. We note however that there are
precedents for such behavior; thus, certain phage hydrolases have been
shown to maintain or even increase their exogenous lytic activity when
the C terminus is truncated (17–19). These findings may have impor-
tant implications for the development of lysins as therapeutic agents.

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