Elucidation of the Molecular Recognition of Bacterial Cell Wall by Modular Pneumococcal Phage Endolysin CPL-1*‡§

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Pneumococcal bacteriophage-encoded lysins are modular proteins that have been shown to act as enzymatic antimicrobial agents (enzymbiotics) in treatment of streptococcal infections. The first x-ray crystal structures of the Cpl-1 lysin, encoded by the pneumococcal phage Cp-1, in complex with three bacterial cell wall peptidoglycan (PG) analogues are reported herein. The Cpl-1 structure is folded in two well defined modules, one responsible for anchoring to the pneumococcal cell wall and the other, a catalytic module, that hydrolyzes the PG. Conformational rearrangement of Tyr-127 is a critical event in molecular recognition of a stretch of five saccharide rings of the polymeric peptidoglycan (cell wall). The PG is bound at a stretch of the surface that is defined as the peptidoglycan-binding sites 1 and 2, the juncture of which catalysis takes place. The peptidoglycan-binding site 1 binds to a stretch of three saccharides of the peptidoglycan in a conformation essentially identical to that of the peptidoglycan in solution. In contrast, binding of two peptidoglycan saccharides at the peptidoglycan-binding site 2 introduces a kink into the solution structure of the peptidoglycan, en route to catalytic turnover. These findings provide the first structural evidence on recognition of the peptidoglycan and shed light on the discrete events of cell wall degradation by Cpl-1.

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† The on-line version of this article (available at http://www.jbc.org) contains supplemental Tables 2 and 3 and Figs. 1 and 2.

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The abbreviations used are: PG, peptidoglycan; MurNAc, N-acetylmuramic acid; 2S2P, GlcNAc-MurNAc-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala); 2S5P, GlcNAc-MurNAc-L-Ala-D-Glu-D-Man-L-Lys-L-Ala-D-Ala); PGBS1, peptidoglycan-binding site 1; PGBS2, peptidoglycan-binding site 2; PGPs, peptidoglycan recognition proteins; r.m.s.d., root mean square deviation; CBP, choline-binding protein.

Streptococcus pneumoniae is one of the most common and important human pathogens, which causes serious life-threatening diseases such as acute otitis media, pneumonia, sepsis, and meningitis. Pneumococcal infections are associated with high morbidity and mortality, especially among children, the elderly, and the immune-depressed patients. The widespread emergence of antibiotic resistance and the lack of highly effective pneumococcal vaccines against all serotypes of this organism give urgency to elucidation of the molecular processes involved in its pathogenicity (1, 2).

The peptidoglycan (PG) scaffold of the bacterial cell wall is a repeating GlcNAc-N-acetylmuramic (MurNAc) disaccharide (GlcNAc-(β-1,4)-MurNAc) unit having a pentapeptide attached to the d-lactyl moiety of each MurNAc unit. All known pneumococcal bacteriophages encode an amidase or a lysozyme, which hydrolyzes the PG at the final stage of the phage reproductive cycle, leading to bacterial cell lysis. These enzymes, known collectively as endolysins, have been shown to be highly efficient in killing pneumococci in vitro and can eradicate this organism from the upper respiratory tract or from the bloodstream of mice (3, 4) acting as new antimicrobial agents (i.e. enzybiotics). In addition, Cpl-1 lysin and Pal amidase encoded by phage Dp-1 act in a synergistic manner in a sepsis mouse model (5); this synergy has also been confirmed in in vitro experiments with Cpl-1 and penicillin or gentamicin (6).

Very recently, the creation of a new animal model of otitis media has been reported (7). Using this new mouse model, it has been demonstrated that Cpl-1 could eliminate colonization with S. pneumoniae and prevent the development of otitis media (7). A paucity of information exists presently on the mechanisms of lysis of pneumococcal cell wall by the phage-encoded endolysins at the molecular level.

All known pneumococcal endolysins display a modular structure. In addition to the catalytic module, all but one possesses a choline-binding module (CBM) to facilitate their anchoring to the choline-containing teichoic acid of the pneumococcal cell wall by Modular Pneumococcal Phage Endolysin CPL-1.
mococcal cell wall (8). This CBM is formed by a repeat of about 20 amino acids, found in multiple tandem copies (ranging from 4 to 18) in a large family of surface proteins (144 members identified by Pfam) from Gram-positive bacteria and from their bacteriophages. These proteins, named choline-binding proteins (CBPs) play important physiological functions in pneumococcal virulence (9). Only crystal structures of two complete CBPs, those of Cpl-1 (10) and Pce (11), have been reported.

Cpl-1 belongs to the GH25 family of glycosyl hydrolases and cleaves the β1-4 glycosidic bond between the MurNAc and the GlcNAc residues of the pneumococcal PG. Structural knowledge of how this process takes place in Cpl-1 and in all other members of the GH25 family of glycosyl hydrolases is presently lacking.

Here we report the crystal structures of the native Cpl-1 lysozyme and of a catalytically inactive mutant variant, referred to as Cpl-1E94Q, in complex with cell wall PG analogues. The combination of crystallographic and computational studies has allowed us to gain unprecedented insights into the recognition events that lead to the catalytic turnover processes. This knowledge is central in understanding how pneumococcal envelope is degraded by Cpl-1, but it also sheds light on the important question of how enzybiotics function against pneumococci and other Gram-positive bacteria.

**EXPERIMENTAL PROCEDURES**

**PG Analogues Synthesis**—The PG analogues 2S2P and (2S5P)₂ were synthesized as described earlier (12, 13).

**Expression and Purification of Cpl-1 Endolysin**—The native Cpl-1 lysozyme and its Cpl-1E94Q mutant variant were expressed in *Escherichia coli* DH1 (pCIP100) and *E. coli* DH1 (pCOB7) cells, respectively, and purified from the crude extracts as described (14).

**Crystallization and Data Collection**—Native crystals of Cpl-1 and Cpl-1E94Q were grown using the hanging drop vapor diffusion method, as reported previously (15). Complexes of the wild-type and mutant lysozymes with disaccharide dipeptide GlcNAc-MurNAc-(L-Ala-D-isoGln) (2S2P), the disaccharide pentapeptide GlcNAc-MurNAc-(L-Ala-d-Glu-L-Lys-d-Ala-d-Ala) (2S5P), and the tetrasaccharide dipeptide GlcNAc-MurNAc-(L-Ala-d-Glu-L-Lys-d-Ala-d-Ala)₂ (2S5P)₂ (Fig. 1) were obtained by soaking the crystals of both proteins in solutions containing the PG analogues. Soaking time was 30 min in all cases except for the 2S5P where the time was 18 h; the concentrations for the ligands were 100 mM for the 2S2P and 25 mM for the 2S5P and the (2S5P)₂, GlcNAc and MurNAc monosaccharides, (GlcNAc)₄, (GlcNAc)₆ oligosaccharides, and the PG analogues MurNAc-(L-Ala-d-isoGln) and MurNAc-(L-Ala-d-isoGln-d-Lys-d-Ala-d-Ala) were also tested without success. The x-ray data sets of the wild-type and Cpl-1E94Q mutant in complex with 2S2P were collected up to 2.3 and 2.0 Å resolution, respectively, using the graphite monochromatic CuKα (λ = 1.5418 Å) radiation generated by an Enraf-Nonius rotating anode generator and a MAR345 image plate detector. The x-ray data sets of the Cpl-1E94Q in complex with 2S5P and (2S5P)₂ were measured at the ID29 beamline of the ESRF up to resolutions of 1.8 and 1.7 Å, respectively. Crystals of the four complexes belong to the orthorhombic C222₁ space group with one molecule in the asymmetric unit. The x-ray diffraction data sets were processed and scaled using the programs MOSFLM (16) and SCALA from the CCP4 package (17).

**Structure Determination and Refinement**—All structures were solved by the Molecular Replacement Method. Structures of lysozyme complexes with 2S2P were solved using the program AMoRe (18), whereas those of the Cpl-1E94Q in complex with 2S5P and (2S5P)₂ were solved using the program MOLREP (19). The crystal structure of the native Cpl-1 (10) was used as the initial model. The models were subjected to successive refinement cycles with the CNS program (20) and manual model building used the software package O (21). After this initial round of refinement, the model was further refined by iterative maximum likelihood positional and TLS refinement in REFMAC5 from the CCP4 package (17). Electron density maps of excellent quality were obtained for all ligands. In the complexes with 2S2P, the complete ligand molecule was observed. In the Cpl-1E94Q-2S5P complex, two ligand molecules were seen in the crystallographic structure. Overall, the resulting electron density was of excellent quality except for two amino acid side chains of the linker region between modules (residues 191–200). Description of the atomic composition of the four complexes together with structure determination parameters and refinement statistics are summarized in Table 1.
Docking Studies—The docking studies were carried out with the FlexiDock module of the SYBYL 6.9 suite of programs (Tripos Inc., St. Louis). A PG model was constructed on the basis of the crystallographic complexes, the hexasaccharide tri-pentapeptide (GlcNAc-MurNAc-(L-Ala-D-Glu-L-Lys-D-Ala-D-Ala))₃ (hereafter (2S5P)₃). The structure of Cpl-1 obtained from x-ray was edited and prepared. The (2S5P)₃ model was constructed from the crystallographic complex Cpl-1E94Q-(2S5P)₂ by adding the GlcNAc-MurNAc-(L-Ala-D-Glu-L-Lys-D-Ala-D-Ala) fragment at positions /H₁₁₀₀₂₁ and /H₁₁₀₀₂₂. In all cases, MMFF94 force field (22, 23) together with parameters especially derived for carbohydrates (24) and charges were applied with the use of distance-dependent dielectric constants and conjugate gradient method until the gradient reached 0.005 kcal mol⁻¹ Å⁻¹. Enzyme-ligand complexes were built on the basis of the crystallographic complex and refined using FlexiDock with a genetic algorithm (25) to determine the optimum ligand geometry.

For docking procedure, the protein was considered rigid except the residues involved in the binding site (sphere of 7 Å), whereas the ligands were considered flexible. Several runs of flexidock were performed to obtain a series of model complexes. These complexes were analyzed and clustered in families based on the following: (i) score energy from flexidock results; (ii) agreement between the experimental data and the theoretical model (the interactions were examined with the LPC program (26)); and (iii) free energy of binding (ΔG_bind) calculated with Structural Thermodynamics Calculations version 4.3 (27). The representative conformer from each group was reoptimized.

RESULTS AND DISCUSSION

Crystal Structures of Cpl-1 in Complex with PG Analogues—The Cpl-1 structure is folded in two well defined modules connected by a linker (Fig. 2). The catalytic module consists of an irregular (β/α)₅ β/α₃ barrel where the PG is hydrolyzed. The CBM presents six choline-binding repeats (p₁–p₆) forming a β/α₂-hairpin each and a C-terminal tail of 16 amino acids. The first four repeats (p₁–p₄) are folded in a super-helical arrangement (CI domain, Fig. 2), whereas the other two repeats (p₅–p₆) and the C-terminal tail fold as an antiparallel-like six-stranded β-sheet
(CII domain) that mediates interaction between modules (Fig. 2). The first four repeats provide two canonical choline-binding sites in Cpl-1, analogous to the same architecture displayed in other CBPs, such as Pce (11) and C-LytA (28). An additional choline-binding site, located at the top of the CBM, is formed by three aromatic residues (Trp-210, Phe-218, and Tyr-238).

Structural studies were performed with PG analogues shown in Fig. 1. Superimposition of the structures of the crystallized complexes with that of the native Cpl-1 gave r.m.s.d. values ranging from 0.48 to 0.64 Å.

The substrate-binding cleft of lysozymes and other glycosyl hydrolases can usually accommodate several saccharide units designated as positions $-i$ (the nonreducing end) through $+j$. The saccharide units flanking the scissile glycosidic bond are assigned as positions $-1$ and $+1$. In all Cpl-1 complexes crystallized, the PG analogues were clearly identified in the electron density maps located along a groove leading to the Cpl-1 active site (Fig. 3 and supplemental Fig. 1). This groove, hereafter referred to as the peptidoglycan-binding site 1 (PGBS1), is built by a short loop (residues 125–129) after $\beta5$ and by the big loop (residues 151–173) placed between $\beta6$ and $\beta7$. The muropeptide analogues 2S2P (Fig. 3A) and 2S5P (Fig. 3B) interact through their GlcNAc and MurNAc rings with Cpl-1 at positions $+1$ and $+2$, respectively, whereas the tetrasaccharide analogue (2S5P)$_2$ binds through the three GlcNAc, MurNAc and GlcNAc rings at positions $+1$, $+2$, and $+3$, respectively (Fig. 3C). The fourth saccharide ring in (2S5P)$_2$ is not seen in the density. A smaller electron density was also found near the active site and was modeled as a formate ion from the precipitant solution (supplemental Fig. 1). The formate ion binding is made possible by interactions with Ser-13, Lys-34, and Tyr-41. Remarkably, no substrate atoms were found at positions $-1$ and $-2$, despite extensive crystallization trials with a wide variety of smaller sized analogues (data not shown). The absence of ligand atoms at positions $-1$ and $-2$ can be explained by the steric restrictions imposed by the Cpl-1 crystal packing at these positions (Trp-210 of a symmetry-related molecule is ensconced at the putative $-2$ position).

Considering the similarity of structural results among all three complexes, we discuss here the complex of the protein with the largest PG analogue (Cpl-1-(2S5P)$_2$). The substrate analogue binds to the enzyme via two GlcNAc and one MurNAc residues, along with its associated peptide stem. No electron density was observed for either the terminal MurNAc saccharide ring or its stem peptide, but the remainder of the structure of the (2S5P)$_2$ molecule is well defined in the electron density map sequestered in the PGBS1 (Fig. 3C and Fig. 4A).
The GlcNAc ring at position +1 is primarily stabilized by hydrogen bonds with the hydroxyl group of Tyr-125 and the carboxylic group of the catalytic Glu-94 (Gln in the inactive mutant), as well as by hydrophobic interactions with Ala-151 and Tyr-153 side chains (Fig. 4B). The MurNAc ring in position +2 makes a stacking interaction with Tyr-127. In addition, a polar interaction exists between the hydroxyl group of Tyr-127 and C-6 of the MurNAc. The peptide stem is tightly packed with a Cpl-l loop (residues 151–154). No electron density was observed for the last two amino acids (d-Ala-d-Ala) of the ligand. We add that the solution NMR structure of (2S5P)2 had revealed the L-Lys-d-Ala-d-Ala portion of the stem peptide totally devoid of structure, consistent with it being a mobile element (29). This mobility would appear to be true also for the d-Ala-d-Ala portion of the ligand in complex with Cpl-1 and hence its lack of observation in the electron density map.

A Model of Cpl-1 in Complex with a Larger Peptidoglycan Segment—Crystallization trials failed to provide information on peptidoglycan binding at positions +2 to +4. Meroueh et al. (29) recently reported the NMR solution structure for (2S5P)2, which revealed that the saccharide backbone of the structure produced dihedral angles for the three consecutive glycosidic bonds that repeated themselves, producing a highly regular right-handed helix for the sugar backbone. Interestingly, this same right-handed helix is also observed in the three saccharide rings of the Cpl-1-(2S5P)2, superimposable to the conformation determined in solution. Edified by this knowledge, we attempted to model a larger structure for the peptidoglycan into the active site of our crystal structure with occupancy at positions +2 to +4. The model revealed contacts of the MurNAc at position −2 and the GlcNAc at −2, a region (hereafter referred to as the peptidoglycan-binding site 2 (PGBS2)) formed by the active site and the -hairpin region (residues 34–45), where the formate ion was found in the crystallographic complexes (Fig. 4A).
Asp-92 was also seen (Fig. 4C). The stem peptide of the MurNAc at position 1 experiences a network of hydrophobic and polar interactions (detailed in supplemental Tables 2 and 3). These interactions force the 1 sugar to acquire a boat conformation that introduces a kink in the PG right-handed helix. Interactions with GlcNAc at position 2 are predicted to take place mainly through a stacking interaction with His-14.

Implications for the Catalytic Mechanism of Cpl-1—Hydrolitic action of lysozymes takes place via a general acid/base mechanism that requires two acidic amino acids, one behaves as the proton donor/acid and the other behaves as the nucleophile/base that promotes the hydrolytic attack by a water molecule. The previously proposed (10) electrophilic and nucleophilic residues are Glu-94 and Asp-10, respectively, and are fully conserved within the GH-25 family. As seen by the short distances, Asp-182 and Asp-92 make low barrier hydrogen bonds (30) with the catalytic residues (2.42 Å for Asp-10 to Asp-182, and 2.59 Å for Asp-92 to Glu-94), with potential implications for the enzymatic reaction. Furthermore, the distortion of the conformation of the substrate at position −1 to a higher energy species (boat versus chair conformers) generates the kink in the substrate, which is likely to be a high energy species en route to the transition state for the reaction, as has been also described for other glycosyl hydrolases (31).

The distance of 9.5 Å between the two catalytic residues (Asp-10 and Glu-94) in the Cpl-1 structure is consistent with that expected for an inverting enzyme (9.0 and 9.5 Å for inverting α- and β-glycosidases, respectively, and 4.8 and 5.3 Å for retaining α- and β-glycosidases, respectively) (32, 33). The distance of 7.2 Å between Asp-10 and the anomic carbon of the −1 sugar in the model of Cpl-1-(255P)3 complex could accommodate a water molecule between them. Asp-10 would activate the water molecule for attack at the glycosidic bond. The side chain of Glu-94 is at H-bond distance of the oxygen of the β1–4 bond; hence, it is poised for the transfer of a proton to the leaving group oxygen, making the hydrolytic process possible.

Peptidoglycan-binding Mechanism—No significant structural differences were found between the complexes with the native Cpl-1 or with the catalytically inactive mutant Cpl-1E94Q upon substrate binding as deduced by their low r.m.s.d. values. However, the structure of the complex with the ligands reveals a profound conformational change of the side chain of Tyr-127 (Fig. 5). This movement involves a change in the values of the two side chain torsion angles (χ1 and χ2) of Tyr-127, which experience displacements of Δχ1 = 101° and Δχ2 = 25° from the native to the complexed state. In the absence of substrate, the side chain of Tyr-127 is placed in the center of the substrate-binding cleft, making polar interactions with Tyr-125 and Tyr-153, via their hydroxyl groups, and a few water molecules (Fig. 5A). In this conformation, Tyr-127 completely blocks access of the substrate to the active site at position +1. Interaction of the active site with the PG chain facilitates repositioning of the tyrosine side chain to a hydrophobic pocket created by Phe-130 and Pro-129 and places it in a suitable conformation for interacting with the MurNAc at position +2 (Fig. 5B). The displacement of Tyr-127 from the central position in the active site and its subsequent new interaction with MurNAc at position +2 must be pivotal to catalysis. It is not merely that the repositioning of Tyr-127 makes room for binding of PG, but it also would lower the energy barrier for the hydrolytic reaction by its interaction with the substrate, even though this electrostatic interaction is one saccharide downstream from the seat of the reaction. Other observations of note are that substrate analogues lacking the

FIGURE 5. Substrate channel before and after ligand binding. In the absence of the PG ligand Tyr-127 blocks the entrance to the active site (A); upon ligand binding, Tyr-127 repositions itself to allow access to the substrate (B). Hydrogen bonds are drawn as dashed lines.
GlcNAc at +1 position or the peptide moiety (of at least two amino acids) on MurNAc at position +2 fail to bind to Cpl-1. These specific interactions at positions +1 and +2 might be crucial in triggering the Tyr-127 movement as a gatekeeper to the active site and to the onset of catalysis.

The crystallographic complexes showed that Cpl-1 interacts well with the first two amino acids of MurNAc at position +2 and to a lesser extent with the third (L-Lys). The expanded computational model reveals that the peptide moiety of the MurNAc at position +1 should be nicely anchored within PGBS2 region (Fig. 4, A and C). This interaction network can explain why mutation of Glu-37 (a residue within the PGBS2 site) to alanine or lysine results in a drastic loss of the catalytic activity, whereas the E37Q mutant variant still retained 67% of activity (34).

**Substrate Recognition in the GH-25 Family—Sequence comparison among the GH-25 family and inspection of crystal structures of Cpl-1, Cellosyl, the muramidase encoded by Streptomyces coelicolor (35), and the very recently reported structure of the catalytic domain of PlyB, a lysin from the phage BcpI (36), allowed us to analyze whether the proposed peptidoglycan-Cpl-1 binding model can be applied to other family members. Besides the catalytic residues, several residues interacting with the MurNAc residue at position −1, such as Tyr-125 and Tyr-59, are also conserved in the GH-25 family (Fig. 6A). When the PGBS2 region of Cpl-1 is compared with the...
equivalent regions in Cellosyl and PlyB_cat, one concludes that
the type of amino acids implicated in recognition at position 
1 by Cpl-1 (Lys-34, Glu-37, Tyr-41, Tyr-59, Phe-61, and Arg-63;
Cpl-1 numbering) is highly conserved (Fig. 6, A and B). His-14,
the side chain of which is proposed to be critical in the stabiliz-
ation of GlcNAc at the \(-2\) position, is either conserved or
conservatively replaced by an aromatic residue, tyrosine or
tryptophan, as in the case of Cellosyl and PlyB_cat (Trp-13 and
Trp-10, respectively). This reinforces the essential role of this
stacking interaction in peptidoglycan recognition at position 
\(-2\) by the GH-25 family.

Concerning the PGBS1 region, the aromatic residue at posi-
tion 153 (Cpl-1 numbering) stabilizing GlcNAc at \(+1\) is system-
atically conserved. However, the critical Tyr-127, whose
motion opens up the active site, is seen only in lysozymes
encoded by pneumococcus and its bacteriophages. However, in
other GH-25 family members, for which structures have been
reported, the Tyr-127 position is also occupied by an aromatic
residue (His-101 in Cellosyl and His-124 in PlyB) (Fig. 6B).
Interestingly, in the case of PlyB, the side chain of His-124 is
placed centrally in the binding site, implying the possibility for
a similar displacement upon substrate binding seen for Tyr-127
in Cpl-1.

Molecular Recognition of Pneumococcal Cell Wall—The
structure of the peptidoglycan bound to Cpl-1 at positions \(+1\),
\(+2\), and \(+3\) is virtually identical to the solution structure of the
fragment, whereas the enzyme introduces a kink in the pepti-
doglycan backbone at positions \(-1\) and \(-2\) (Fig. 7, A and B). It
would appear that Cpl-1 recognizes the solution structure of the
peptidoglycan (at peptidoglycan-binding site 1) as it distorts
the polysaccharide backbone (at peptidoglycan-binding site 2)
en route to turnover.

It has been suggested that PG glycosyltransferases are pro-
cessive enzymes, meaning that they catalyze multiple rounds of
coupling without releasing the elongating product. This model
for processive glycosyl chain synthesis has been documented
Pneumococcal Cell Wall Degradation by Cpl-1

for the PG glycosyltransferase domain of *Aquifex aeolicus* recently (37), whose three-dimensional structure resembles that of the bacteriophage λ-lysozyme. Although no experimental information on processivity by Cpl-1 is available, the, structurally related enzyme Cellosyl from *S. coelicolor* exhibits a processive mechanism. The experimental demonstration of processivity waits for Cpl-1, but the structure reported herein argues that as the hydrolytic reaction proceeds at the juncture of the peptidoglycan-binding sites 1 and 2, the enzyme might move along the backbone of the peptidoglycan in performing the catalytic process repeatedly (Fig. 7B).

The aforementioned right-handed helical conformation of the peptidoglycan polysaccharide backbone produced a 3-fold symmetry in the molecule (29). Each peptidoglycan strand is cross-linked to a neighboring strand to give a continuous covalent network for the cell wall that covers the entire bacterium. The 3-fold symmetry presents the opportunity for cross-linking to a maximum of three neighboring strands but requires a minimum of two, which led to the proposed honeycomb pattern for the cell wall (29). This model accommodates openings of as small as 70 Å or larger in the cell wall. For example, the absence of a single peptidoglycan strand in the perfect honeycomb generates an opening of 120 Å. The model of Cpl-1 binding to the peptidoglycan based on the x-ray structure of the enzyme and the solution NMR structure of the peptidoglycan depicted in Fig. 7B can be directly accommodated with this honeycomb pattern in openings of 120 Å (when one strand of peptidoglycan is missing) or larger (Fig. 7C).

A Common Peptidoglycan Recognition Pattern?—In the fight against infections, multicellular animals have adapted their immune system to recognize microorganisms by detecting conserved structures. This recognition constitutes the first line of defense by the host. Peptidoglycan molecules are located on the surface of all Gram-positive bacteria and therefore constitute structures. This recognition constitutes the first line of defense for the PG glycosyltransferase domain of *Aquifex aeolicus* and the native pneumococcal phage Cpl-1 lysin in complex with three cell wall PG analogues. This structural information provides for the first time insights into how the cell wall is recognized by this enzyme and defines the incremental steps involved in the catalytic events that lead to hydrolytic fragmentation of the cell wall. In light of the fact that phage lysins such as Cpl-1 have been documented to have antibacterial effects in animal models for infections (the word “enzymatic” has been coined) our report defines the mechanism of this antibacterial effect.

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Pneumococcal Cell Wall Degradation by Cpl-1