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High-Resolution Crystal Structure of MltE, an Outer Membrane-Anchored Endolytic Peptidoglycan Lytic Transglycosylase from *Escherichia coli*

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Supporting Information

ABSTRACT: The crystal structure of the first endolytic peptidoglycan lytic transglycosylase MltE from *Escherichia coli* is reported here. The degradative activity of this enzyme initiates the process of cell wall recycling, which is an integral event in the existence of bacteria. The structure sheds light on how MltE recognizes its substrate, the cell wall peptidoglycan. It also explains the ability of this endolytic enzyme to cleave in the middle of the peptidoglycan chains. Furthermore, the structure reveals how the enzyme is sequestered on the inner leaflet of the outer membrane.

The cell wall is recycled during the course of the normal doubling of bacterial cultures as well as in response to damage, which might be inflicted by antibiotics.[1,2] The major component of the cell wall is the peptidoglycan (PG), the neighboring strands of which undergo cross-linking for assembly of the functional cell wall. PG is comprised of alternating β-1,4-linked *N*-acetylmuramic acid (MurNAc) and *N*-acetylglucosamine (GlcNAc) residues. A unique peptide stem, the site of cross-linking, is appended to lactyl groups of the MurNAc residues.[1] The reaction of lytic transglycosylases (LTs) is unusual in that the β-1,4-glycosidic bond between MurNAc and GlcNAc of PG is cleaved in an initial step that is believed to go through an oxocarbonium species, which in turn entraps the C6-hydroxyl moiety of MurNAc. This substrate-assisted catalysis leads to the formation of metabolite **1** (GlcNAc-anhMurNAc-L-Ala-D-Glu-m-Dap-D-Ala-D-Ala), which produces a nonreducing 1,6-anhydromuramyl residue. Metabolite **1** is taken up by the permease AmpG,[3] and once in the cytoplasm, it undergoes a series of reactions that ultimately lead to the formation of lipid II, which is translocated to the surface of the bacterium for de novo synthesis of PG. Of the seven known *Escherichia coli* LTs, six are anchored to the outer membrane (MltA−MltF) and one is soluble (Slt70).[4] A comparison of genes encoding LTs allowed their classification into four families.[5] LT family 1 includes Slt70 and MltC−MltF, whereas MltA and MltB represent families 2 and 3, respectively.[6,7] Most LTs appear to act as exolytic enzymes, releasing metabolite **1** from the ends of the glycan strands. The only exception in *E. coli* would appear to be MltE, which is proposed to be an endolytic enzyme. This is the ability to fragment the peptidoglycan strand in the middle, which ultimately would lead to the formation of **1** by the action of other LTs on products of the reaction of MltE.[8] Structural research on LTs has so far resulted in the X-ray structures of the 70 kDa soluble Slt70,[9] the 36 kDa soluble Slt35 of *E. coli*,[7,10] and the 38 kDa MltA.[6,11,12] The Slt70 structure is built up of three distinct domains (U, L, and C domains), which are all rich in α-helices. The N-terminal domain is packed in a U-shaped conformation and connected to an L domain forming a closed ring with a large central opening.[11] The C-terminal domain is the catalytic one and is packed on top of this ring, interacting with both L and U domains. This C domain has a globular structure as the goose-type lysozyme.[11] Structural studies of Slt70 with peptidoglycan fragments[9] revealed the presence of six saccharide binding sites in the active site groove. Slt35 is a fully active, soluble form of the 40 kDa membrane-anchored MltB. The structure of Slt35 of LT family 3 shows three domains named the α, β, and catalytic domains[7]. The catalytic domain contains Glu162, which coincides with the catalytic Glu478 of Slt70 and Glu73 of goose-type lysozyme after superposition. Both Slt35 and Slt70 are exomuramidases that require the peptide side chains in peptidoglycan for activity.[13,14] It is believed that the N-terminal, doughnut-shaped domain of Slt70 together with the presence of a specific binding site for 1,6-anhydromuropeptide product and the "exo loop" of Slt35 (residues 99−108) impose this exomuramidase activity.[7,15] MltA (LT family 2), whose crystal structures from *E. coli* and *Neisseria gonorrheae* have been reported,[11,12] presents a catalytic domain completely different from that of lysozyme.[16] For LT family 1, only the three-dimensional structure of soluble exolytic Slt70 has been reported. We report here the first high-resolution (2.0 Å) crystal structure for the endolytic MltE.

Multisolution phasing through a search of small fragments derived from the catalytic module of Slt70[15] combined with density modification was successfully applied using ARCIMBOLDO.[17] Two monomers were built in the asymmetric unit [root-mean-square deviation (rmsd) of 0.19 Å for all Cα atoms], and refined to 2.0 Å resolution. MltE has a globular structure with 10 α-helices (Figure 1A). Its overall fold is similar to that of goose-type lysozyme. Utilizing the Dali server,[18] the closest

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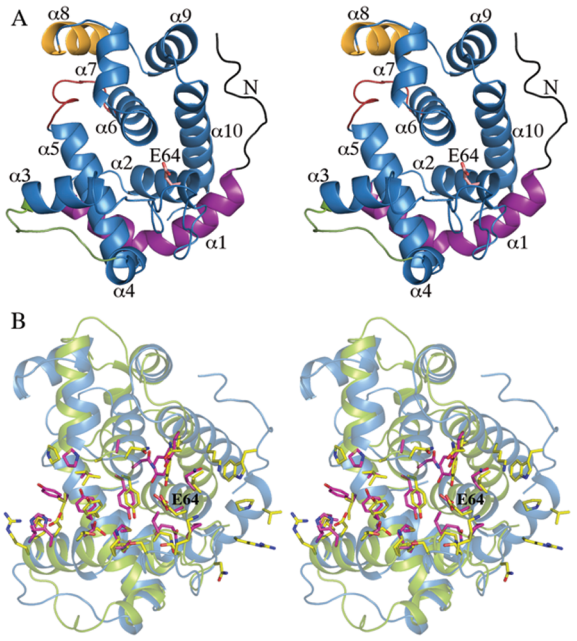

Figure 1. (A) Three-dimensional structure of MltE. The main structural differences with Slt70 are colored black (N loop), magenta (α1 helix), red (the loop connecting α5 and α6), and yellow (α8). The catalytic Glu64 is labeled. (B) Structural superimposition of MltE (blue ribbons) and the catalytic domain of Slt70 (green ribbons). Side chains building active sites are represented as sticks (yellow for MltE and pink for Slt70).

structural models of MltE are the catalytic module of Slt70[15] [rmsd of 3.1 Å for 161 Cα atoms (see Figure S1A of the Supporting Information)], the G-type lysozyme[19] (rmsd of 2.6 Å for 144 Cα atoms), and the Slt35 lytic transglycosylase[7] (rmsd of 2.5 Å for 127 Cα atoms). Despite the overall structural homology, significant differences exist between MltE and the catalytic domain of Slt70 (Figure 1B and Figure S1 of the Supporting Information). This is relevant as a high degree of sequence homology is observed in Slt70s[15] and MltEs (Figure S1C of the Supporting Information). Among structural differences, three of them are critical for explaining specific features in MltE: the presence of an N-terminal loop (residues 19−30) and the long α1 helix (residues 30−50) that is markedly longer than the corresponding helix in Slt70 and the presence in MltE of a new α-helix (α8) presenting a basic patch of surface on one side of the protein (Arg161, Lys162, Lys163, and Lys167). While the first two differences are important for the endolytic character of MltE, the orientation of the N-terminal region and the new α8 have implications for interactions with the outer membrane by MltE (see below). As revealed in the MltE crystal structure, the long N-terminal loop (residues 19−30), not present in the Slt70 structure, is strongly stabilized through interactions with the core of the protein (see Table S2 of the Supporting Information) and provides a rigid scaffolding that connects the point of membrane anchoring [acylated Cys15, discussed below (Figure 2)] with the active site. In fact, Trp27, a residue within the N-terminal loop, is part of the active site (Figure 1B and Figure S2 of the Supporting Information). As indicated earlier, MltE is the only LT of *E. coli* with peptidoglycan endolytic activity. As such, the structure reveals it to have an extended active site that binds at least eight saccharides of the peptidoglycan chain (vide infra) as obtained by molecular dynamics (MD) simulations. The substrate-binding cleft of lysozymes and other glycosylhydrolases accommodate several saccharide units at subsites designated as positions $-i$ (the nonreducing end) through $+j$ (in the other direction). The saccharide units flanking the scissile glycosidic bond are designated as positions −1 and +1. The ellipsoid shape of MltE with its two lobes is linked by long helix α5. The active site is sequestered in a deep groove that spans the two lobes within the −4 to +4 saccharide-binding subsites (Figure 2) versus the −4 to +2 saccharide-binding subsites reported for Slt70.[9] The peptide-binding site at position −1 is formed by a shallow groove between α7 and the loop connecting α9 and α10 (Supporting Information), similar to what is found for St70.[15] The site at position −3 is formed by the groove between α3 and α5. Another point of distinction concerns helix α1. This helix (residues 30−50) is longer (20 residues) than the corresponding helix in Slt70 (8 residues) and provides an extension of the substrate-binding site up to subsite +4. In addition, the loop connecting α5 with α6 creates a groove rich in acidic amino acids at the backside of the substrate-binding site (Figure 1A and Figure S3 of the Supporting Information) whose function is likely glycan stabilization of neighboring cross-linked PG strands. The last significantly distinct feature is the presence of an additional α-helix (α8). This helix comprises a cluster of basic residues (Figure 2) that forms, together with Arg182 (from α9) and Lys19 (from the N-terminal loop), a membrane-interacting surface near the acylated Cys15 (Figure 2A).

Glu64 is the catalytic residue at subsite −1 based on its equivalence in position to the catalytic glutamic acid in Slt70 (Figure 1B). The acidic form of Glu64 protonates the glycosidic oxygen that departs from the scissile bond in the substrate to give rise to the intermediary oxocarbonium ion. The now-deprotonated Glu64 would promote the C6-hydroxyl in the formation of the 1,6-anhydromuramyl moiety of the product.[16] The three-dimensional structure of MltE and MD simulations reveals key insights into its unique endolytic activity, beyond the aforementioned expansive active site that spans subsites −4 to +4 for binding to the polymeric substrate. The active site surface accommodates the 3-fold symmetric right-handed helical structure of the peptidoglycan, as determined previously by NMR[20] (Figure S3 of the Supporting Information). It has binding sites for the extended peptide stems that would be appended to the MurNAc moieties, in agreement with the peptide stem requirement for the activity of some LTs.[15] The sites could accommodate the peptides regardless of whether they are cross-linked to a neighboring peptidoglycan. Potential steric encumbrance by such cross-linking would not appear to make a difference. This, in large measure, is likely due to MltE being the only single-domain LT in *E. coli*. The second step reaction of the enzyme, which leads to the formation of the 1,6-anhydroMurNAc bicyclo ring, leads to the switch of all substituents from the equatorial to the axial positions. The conformational influence of this switch is profound, but especially so as far as the peptide moiety is concerned. It has been suggested that the Slt70 active site actually has a surface for binding of the peptide in the 1,6-anhydroMurNAc at subsite +2, but a corresponding peptide-binding site is not seen in the structure of MltE (Figures S4 and S5 of the Supporting Information). This subsite in Slt70 has been postulated as being essential for directing product **1** from subsite −1 to subsite +2 after one turnover event, which allows processivity in the function of the exolytic enzymes such as Slt70[15] (Figure S6 of the Supporting Information). The absence of this subsite in MltE abrogates this opportunity,

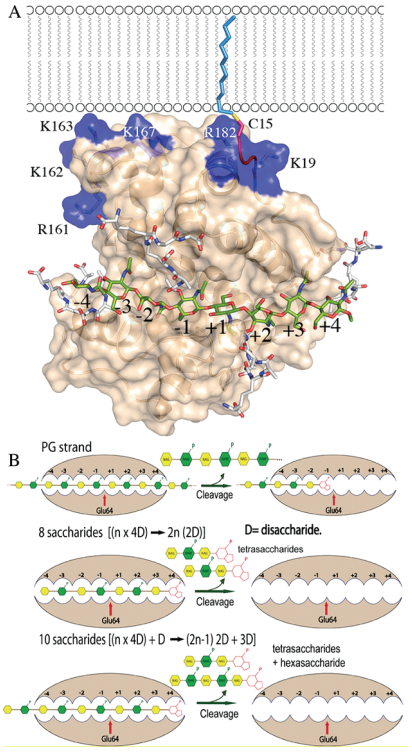

Figure 2. (A) MltE could accommodate saccharides at sites −4 to +4 (and also the associated peptides attached to the MurNAc moieties), consistent with the solution NMR structure for the peptidoglycan (shown in capped sticks). Docked saccharide rings and peptide stems from PG are colored differently (carbon atoms colored green for saccharides and white for peptides). N-Lipidated Cys15, the membrane anchor, is shown as a red coil. Some residues within the basic patch are labeled. (B) Scheme showing the proposed mechanism for endotransglycosylase MltE. Because MltE has eight sugar-binding sites and there are no other structural domains, MltE can cleave in the middle of the polymeric substrate. Four disaccharides (4D) fill the active site and undergo cleavage. For strands having $n \times 4D$ saccharides, the final products will be tetrasaccharides. For substrates having $n \times 4D + D$ saccharides (as a polysaccharide with 10 monosaccharide units), the final products will be $2n - 1$ tetrasaccharides plus one hexasaccharide. This scheme is consistent with the experimental observations.[8]

whereas the presence of eight saccharide-binding subsites supports the reported catalytic outcome for tetra- and hexasaccharide 1,6-anhydroMurNAc variants of the peptidoglycan as products (Figure 2B). In this vein, activity assays indicated that the tetrasaccharide analogue is not a substrate for MltE (data not shown).

Removal of the signal peptide in MltE by signal peptidase is followed by N-acylation of Cys15 by a fatty acid.[8] The lipid penetrates the inner leaflet of the outer membrane. Whereas the contribution to anchoring by this lipid penetration might be quite significant, the structure of MltE suggests that an electropositive surface near the N-terminus might actually provide the draw to the membrane headgroups and also could contribute to substrate orientation during in vivo catalysis. A cluster of positively charged residues is located on this electropositive surface (Figure 2 and Figure S3 of the Supporting Information). The presence of basic patches has been associated with membrane sensing and binding in BAR domains.[21] The relevance of this finding in the catalytic mechanism of LTs awaits additional structural information about other enzymes.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures for structural determination and molecular dynamics calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

The crystallographic coordinates are deposited in the Protein Data Bank as entry 2y8p.

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