University of Groningen

Structure and reaction mechanism of lytic transglycosylases.

van Asselt, E.

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1999

[Link to publication in University of Groningen/UMCG research database](#)

Summary

An intact cell wall is essential for the survival of the bacterial cell. It gives the cell its shape and stability and it protects the bacterium against the high osmotic pressure inside the cell. To keep the cell wall intact during the life cycle of the bacterial cell, the bacterium uses a large number of cell wall metabolising enzymes, which closely work together. Some enzymes assemble and/or cross-link the building blocks of the cell wall (disaccharide-peptides) into a network of peptidoglycan strands, while other enzymes remove, under strict control, small cell wall fragments to make space for the insertion of new cell wall material during the growth of the cell. Furthermore, some enzymes make pores in the cell wall to allow transport of proteins and DNA. How the activities of the cell wall enzymes are precisely coordinated is not completely clear. But if the fine-tuned interplay between the cell wall enzymes is interrupted by stress factors or inhibitors, the cell wall structure can get damaged to such an extent that the high internal pressure can cause cell lysis.

Many pathogenic bacteria, including *Yersinia pestis* (bubonic plague), *Bordetella pertussis* (whooping cough), *Neisseria meningitidis* (meningitis), *Neisseria gonorrhoea* (venereal disease), *Salmonella typhimurium* (food poisoning), *Helicobacter pylori* (gastric ulcer) and *Escherichia coli* (diarrhoea), have cell walls, which are very similar and consist of the same building blocks. Because the cell wall is essential for the bacteria, and the cell wall building blocks are unique to bacteria, the enzymes involved in the maintenance of the cell wall are an interesting group of targets for antibiotics. For example, penicillin and other β-lactam derivatives inhibit enzymes involved in cross-linking of the peptidoglycan, which results in disruption of the cell wall and eventually cell lysis. Unfortunately, many pathogenic bacteria have developed resistance against these antibacterials and therefore, new inhibitors are required to combat bacterial infections.

The cell wall of *Escherichia coli* is located between the inner and outer membrane and consists of linear polysaccharide chains of alternating *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) residues cross-linked by short peptide bridges. The peptide chains are attached to the MurNAc *D*-lactate groups and consist of 3 to 5 *L*- and *D*-amino acid residues. The pentapeptide consists of *L*-alanyl-*D*-glutamyl-*m*-diaminopimelyl-*D*-alanyl-*D*-alanine (*L*-Ala-*D*-Glu-*m*-Dap-*D*-Ala-*D*-Ala). During the cell cycle, new peptidoglycan is cross-linked to the cell wall via peptide bonds by specific enzymes. These enzymes can be inhibited by penicillin, because penicillin mimics the *D*-Ala-*D*-Ala peptide bond and binds irreversible to these penicillin-binding proteins. Cell lysis can therefore take place.

In contrast, lytic transglycosylases do not act on the peptide bonds, but they cleave the glycosidic bond between a MurNAc residue and a GlcNAc residue in the peptidoglycan. This

step is accompanied by the formation of a so-called 1,6-anhydrobond between the C1 carbon atom and the O6 oxygen atom of the MurNAc sugar (anhMurNAc). The peptide side chains of the 1,6-anhydromuropeptide products (GlcNAc-anhMurNAc-peptides) vary from 3 to 4 residues and sometimes they are still cross-linked to the peptidoglycan polymer. This transglycosylation reaction is unique to bacteria and inhibition of lytic transglycosylases seems to enhance the efficacy of β-lactam antibiotics. Therefore, the lytic transglycosylases are interesting targets for drug design. Knowledge of their three-dimensional structures at atomic level could be helpful for such a drug design approach.

This thesis describes structural research of two lytic transglycosylases from *E. coli*, Slt35 and Slt70, using X-ray crystallography. Although both enzymes show large differences in size and amino acid sequence, their structures have a domain with a similar fold in which the active site is located. In the active sites, several substrates have been bound to gain insight in the interactions of the enzymes with peptidoglycan and in their reaction mechanisms.

Chapter 1 gives an overview of the present knowledge of the cell wall structure and the bacterial lytic transglycosylases of *Escherichia coli*. Furthermore, the structures and reaction mechanisms of other enzymes that can cleave the polysaccharide chains of peptidoglycan are discussed.

Chapter 2 describes the crystallisation and structure elucidation of Slt35. Rod-shaped protein crystals were obtained which diffracted to 1.7 Å resolution. Crystals were subsequently soaked in solutions with mercury, lead or osmium compounds. Due to the binding of the heavy atoms to Slt35, the diffraction patterns of these crystals were sufficiently altered to calculate the distribution of electrons in the crystal at 2.5 Å resolution. Then, a new and powerful method in protein crystallography, called wARP, was successfully applied to improve the electron density map and to extend the resolution to 1.7 Å. Using the wARP method, the building of the three-dimensional structure of Slt35 was substantially accelerated. Slt35 was the first protein of unknown structure to which the wARP method was successfully applied.

Chapter 3 describes the refined crystal structure of Slt35 at 1.7 Å resolution and a complex of Slt35 with a GlcNAc molecule at 2.45 Å resolution. The enzyme consists of three domains named the alpha, beta and core domains. The alpha domain contains an N-terminal subdomain (residues 40-98) and a hairpin subdomain (residues 170-215), which consist of 5 α-helices and 2 short β-strands. The beta domain has a five-stranded antiparallel β-sheet flanked by a short α-helix. The core domain is sandwiched between the alpha and beta domains and contains 8 α-helices. Despite the lack of significant sequence homology, the core domain of Slt35 resembles the fold of the catalytic domain of Slt70. Glu162 of Slt35 superimposes on the catalytic acid/base of Slt70. Mutation of Glu162 into a glutamine

residue yielded a completely inactive enzyme, in support of a catalytic role of Glu162. Glu162 is located in a deep cleft in the core domain, where also a GlcNAc molecule could be bound. Furthermore the core domain has a single metal ion-binding site that shows high homology to EF-hand calcium-binding sites, which are often observed in eukaryotic proteins. The residues of the metal-ion binding site are conserved in all Slt35/MltB lytic transglycosylases from various bacteria, which suggests an important role for the metal ion-binding site.

Chapter 4 focuses on the nature of the metal ion-binding site and its effect on the thermostability of Slt35. Two diffraction data sets were collected from a single crystal after it was soaked in a solution with either sodium or calcium ions. Analysis of the electron density maps clearly showed that both ions can bind to the protein. However, heat-induced unfolding studies with Slt35 in the presence of various metal ions subsequently showed that the thermostability of Slt35 is only increased in the presence of calcium, even if a 100-fold excess of sodium ions is present. This shows that calcium is the natural ion for the metal-ion binding site.

Chapter 5 analyses the interactions of Slt35 with two peptidoglycan fragments and a glycopeptide inhibitor. The peptidoglycan fragments were the disaccharide $(GlcNAc)_2$ and the murodipeptide GlcNAc-MurNAc-*L*-Ala-*D*-Glu. The glycopeptide was the lytic transglycosylase inhibitor bulgecin A, which consists of a 4-O-sulfonyl-GlcNAc residue, and a *L*-proline ring with a taurine residue. Two murodipeptides were found at adjacent sides of the catalytic acid/base Glu162 in the active site. Bulgecin A binds in an extended conformation in the active site cleft and probably mimics an oxo-carbonium ion intermediate. A ternary complex of Slt35, bulgecin A and $(GlcNAc)_2$ shows how the GlcNAc sugar in site +1 might shield this intermediate from the solvent to prevent hydrolysis. The complexes suggest contributions to catalysis from Glu162, Ser216 and Asn339, which residues are conserved among the Slt35/MltB lytic transglycosylases.

Chapter 6 gives a detailed description of the high resolution structure of Slt70. Initially, the Slt70 crystals only diffracted to 2.7 Å resolution, but using cryo-cooling and synchrotron X-ray radiation, diffraction to 1.65 Å resolution could be obtained. The improved model shows, beside the 618 amino acid and almost 1000 water molecules some small ions and molecules: 13 sulfate ions, one acetate ion and 7 glycerol molecules. Slt70 has three domains named the U-, L- and C-domains. The N-terminal and horse shoe-shaped U-domain (residues 1-360), the UL-loop (residues 361-377) and the linker L-domain (residues 378-450) form a ring of 28 α-helices, which are ordered into an α-superhelix. The fold of the superhelix is characterised by two layers of helices, in which the helices are parallel within the same layer, but antiparallel to the helices in the opposite layer. Especially alanine, leucine

and arginine residues are involved in the helix-to-helix packing. This is in contrast to the 9 α-helices of the catalytic C-domain, where mostly alanine and tyrosine residues take part in the helix-to-helix packing. The fold of C-domain resembles the earlier mentioned Slt35 core domain and the goose-type lysozyme structure. In a deep groove of the C-domain, Glu478 acts as the catalytic acid/base of Slt70. In addition, the binding of the 1,6-anhydromurotripeptide GlcNAc-anhMurNAc-*L*-Ala-*D*-Glu-*m*-Dap shows the presence of one peptide-binding site at the interface of the U-, L- and C-domains.

Chapter 7 finishes this thesis with concluding remarks and recommendations for future research on lytic transglycosylases.

The elucidation of the Slt35 structure has contributed to the development of a new and effective method (wARP) to accelerate the building of a new protein structure. In addition the crystal structures of Slt35 and Slt70, and of their complexes with peptidoglycan fragments and inhibitors have given more insight in their structures, interactions with the peptidoglycan and reaction mechanism. This information may be of help in the design of lytic transglycosylase inhibitors.