The hole story. Structure and function of the 70 kDa soluble lytic transglycosylase from escherichia coli

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Summary

Structural integrity of the bacterial cell wall is of vital importance for the prokaryotic cell. Without a rigid, intact cell wall, bacteria are no longer able to resist the high osmotic pressure inside their cells: bulges form at the cell surface and, eventually, the bacteria will burst. The strength and rigidity of the cell wall is defined by a unique molecular mesh known as murein or peptidoglycan. This is a heteropolymer built up of linear polysaccharide strands, cross-linked by short peptide chains, forming a network-like structure that surrounds the cell as one giant macromolecule. The polysaccharide (glycan) strands are composed of two alternating sugar residues, *N*-acetylglucosamine (GlcNAc) and *N*-acetyl-muramic acid (MurNAc), which are connected by β-1,4-glycosidic bonds. The peptides are linked to the glycan chains via the lactyl group of the MurNAc residues and contain a small number of both *L* and *D*-amino acid residues. A typical peptide in the cell wall peptidoglycan of *Escherichia coli* consist of *L*-alanine-*D*-isoglutamyl-*m*-diaminopimelyl-*D*-alanine (*L*-Ala-*D*-Glu-*m*-Dap-*D*-Ala).

The integrity and shape of the peptidoglycan during growth and cell division are thought to be preserved by a balanced action of pepidoglycan-synthesising and peptidoglycan-degrading enzymes. It is a challenge to understand the nature of this interplay of enzymes. Such knowledge would be directly applicable for the development of antibiotica. The antibacterial activity of penicillin, for instance, is due to a disturbance in the synthesis of the peptide cross-linkages in the peptidoglycan. Although penicillin is still the most used drug for treatment of bacterial infections, its potency has been severely diminished due to the development of antibiotic resistance in bacteria. This so called "crisis of antibiotic resistance" has revived the research interest in the peptidoglycan metabolic pathways with the objective to identify new targets for the design of novel antibiotics, distinct from the penicillins. Such potential new targets are the lytic transglycosylases from *Escherichia coli*. These are endogenous peptidoglycan-degrading enzymes involved in bacterial cell wall growth and turnover. The lytic transglycosylases catalyse the cleavage of the β-1,4-glycosidic bonds in the peptidoglycan, an activity that is unexploited by most natural and synthetic antibiotics. The activity is similar to that of the lysozymes, a well-studied class of anti-bacterial peptidoglycan-degrading enzymes. However, while the lysozymes cleave the peptidoglycan by hydrolysis of the glycosidic bonds, the lytic transglycosylases accomplish this by catalysing a glycosyl transferase reaction whereby new, 1,6-anhydro bonds are formed in the MurNAc residues. Furthermore, while lysozyme is an endo-muramidase with

general access to the glycosidic bonds in the peptidoglycan network, SLT70 is an exo-muramidase that can cleave the glycosidic bonds only starting from the loose ends of the glycan stands.

This thesis describes the determination of the crystal structure of the 70-kDa soluble lytic transglycosylase from *E. coli*. The three-dimensional structure provides the framework necessary for studying the catalytic properties of the enzyme. As a long-term objective the structure may be used for the design of specific inhibitors and novel antibiotics.

Chapter 1 provides an overview of the current state of knowledge concerning the bacterial peptidoglycan and the lytic transglycosylases. In spite of extensive biochemical and biophysical studies, little is known about the precise mechanisms that play a role in the assembly, growth and turnover of the bacterial cell wall. The physiological role and the reaction mechanism of the lytic transglycosylase are still elusive. The mechanism of action of lysozyme, on the other hand, is rather well defined, and forms a reference for the analysis of SLT70. In the reaction mechanism of lysozyme two amino acids play an important role: a glutamic acid and an aspartate residue. The glutamic acid starts the cleavage reaction by donating a proton to the central oxygen atom in the scissile, glycosidic bond. As an intermediate step, a very labile, positively charged glycosyl oxocarbonium ion is formed, that is stabilised by the negative charge of the aspartate. In the final step of the reaction this oxocarbonium ion is attacked by a water molecule, releasing the cleaved sugar from the active site of the enzyme.

In **chapters 2 and 3** the three-dimensional structure of SLT70 is introduced. The structure was determined by X-ray crystallography at a resolution of 2.7 Å. The SLT70 protein molecule turned out to have an extraordinary shape. Instead of globular, like most proteins, SLT70 is doughnut shaped, with a large hole in the middle. Electron microscopy confirmed the unusual shape of the SLT70 protein. The SLT70 doughnut is built up of three domains. The first 360 amino acid residues form a rather extended U-shaped domain. By a loop of ~20 amino acids this U-domain is connected to the linker or L-domain (70 amino acids). This second domain closes the mouth of the U-domain, thereby forming a central hole of more than 25 Å in diameter. The first two domains of SLT70 contain only α-helices, 27 in total, which are folded into an unusual right-handed superhelix. The superhelix may be described as two neighbouring layers of α–helices; within each layer helices run parallel, while between layers anti-parallel helix pairs are formed. This arrangement is quite unique and differs from that observed in the "standard" 4-α-helix bundle, in which all helix pairs are anti-parallel. So far, only one protein is known with a domain of similar fold: the lipovelline from the eggs of lamprey.

On top of the superhelical ring lies the third, C-terminal domain, such that the central hole remains largely accessible to solvent. This C-domain consists of nine α-helices and a small antiparallel β-sheet. Its fold resembles that of lysozyme, although there is no obvious relationship in amino acid sequence. By site-directed mutagensis and an inhibitor binding study it could be shown that the C-domain contains the active site of SLT70 with Glu478 as the equivalent of the "catalytic" glutamic acid in lysozyme. However, a "catalytic" aspartate is absent in the active site of SLT70. This implies that the lytic transglycosylase is not able to provide the same stabilisation of a possible oxocarbonium intermediate as lysozyme does. This is not unreasonable, though, since the intramolecular glycosyl transferase reaction catalysed by SLT70 probably requires a shorter lifetime of the oxocarbonium ion compared to the hydrolysis reaction catalysed by lysozyme. The function of the U and L-domains in SLT70 is unknown. Probably they are involved in the binding of peptidoglycan. It is also conceivable that the ring structure of these domains forms a steric obstruction that determines the exolytic activity of SLT70.

Chapter 4 describes a detailed structural comparison of the C-domain of SLT70 with the three-dimensional structures of three different lysozymes: chicken-type hen egg-white lysozyme, goose-type swan egg-white lysozyme and phage-type lysozyme from bacteriopahe T4. Each of these lysozymes represents a distinct class in the lysozyme family on the basis of amino acid sequence and catalytic activity. Our investigations revealed that the C-terminal domain of SLT70 defines a novel bacterial class of lysozymes that is characterised by the absence of a "catalytic" aspartate in the active site and a small number of differences in secondary structure elements. The best agreement in three-dimensional structure is found with the goose-type lysozyme, which interestingly, also misses a catalytic aspartate in the active site.

In **chapters 5 and 6** the results of three crystal binding studies of SLT70 are presented. The experiments were carried out by soaking crystals of SLT70 in solutions containing different peptidoglycan-related compounds. The compounds used included a glycopeptide with a GlcNAc residue and a substituted proline derivative, a trisaccharide of only GlcNAc residues and a glycopeptide consisting of a GlcNAc and a 1,6-anhydroMurNAc residue linked to an *L*-Ala-*D*-Glu-*m*-Dap peptide. The first compound is also known as bulgecin and the complex of SLT70 with this specific inhibitor was mentioned before in chapter 2. The second compound is a fragment of chitin, a polymer from the cell wall of yeast. The last compound is a natural reaction product of SLT70. All three compounds bind to the C-domain of SLT70, in a groove that also contains the active site of the enzyme. Analysis of the structures of the three complexes confirms that SLT70, like lysozyme, utilises an extended, peptidoglycan binding site that can accomodate five to six consecutive saccharide

residues. Furthermore, the results show that the enzyme also has a binding site for the peptide moieties in the peptidoglycan. The role of Glu478 as catalytic acid is confirmed by the SLT70 complexes. The SLT70-bulgecin is especially interesting because with its positively charged proline derivative the inhibitor could mimick the conformation of the oxocarbonium reaction intermediate of SLT70.

The results described in this thesis form a basis for further research on the action and function of SLT70. Several questions remain to be answered. What function have the first two domains in the SLT70 doughnut? Which differences in the catalytic centre of SLT70, in addition to the absence of a catalytic aspartate, determine that SLT70 acts as a lytic transglycosylase, instead of a lysozyme. What mechanism enables the SLT70 doughnut to pass the peptide cross-bridges when it moves along the glycan strands. Structure determination and analysis of SLT70 mutants complexed with larger fragments of peptidoglycan are essential for answering these questions. Future research on the structure and function of SLT70 should establish whether the enzyme is suitable for the design of novel antibiotics.