SUMMARY

In the 1930s infectious diseases formed a major threat to public health. The discovery and application of penicillin and other antimicrobial agents was one of the most important measures leading to the control of bacterial diseases. However, extensive use of these antibiotics has led to the emergence of resistant strains. This forces man to develop ever new antibiotics in a lasting race with microorganisms.

At present infectious diseases are still the world's number one cause of death and a crisis in antibiotic resistance is announcing itself. Ever more pathogens appear to be insensitive to ever more antibacterial agents.

Part of the solution to this crisis is on the social level. More stringent rules have to be set up for the use of antibiotics in hospitals and in the community and hygiene and health care in the slums of large cities in both the first and the third world have to be improved. On the other hand the necessity to develop new antibiotics remains. This development takes place mainly using a trial and error approach. For more rational drug design it is essential to learn more about the interaction between antibiotics and their targets.

The targets of penicillin and other β-lactam antibiotics are the penicillin-binding proteins (PBPs). These proteins, which fulfil essential roles in the synthesis of the bacterial cell wall, can be divided into two groups. The high-M_r PBPs are bifunctional enzymes forming the lethal targets of β-lactam antibiotics. The low-M_r PBPs are monofunctional and are therefore much easier to study. Since they show considerable similarity to the high-M_r PBPs they form suitable model systems.

In this thesis the structure function relationship of two low-M_r PBPs of *Escherichia coli*, PBP5 and PBP6, was investigated. Structural and mechanistic data of β-lactamases were used. β-Lactamases, which are related to PBPs, hydrolyse β-lactam antibiotics and are largely responsible for bacterial resistance.

Studying the enzymatic characteristics of a protein and determining its three-dimensional structure using X-ray diffraction, necessitates large quantities of purified material. Chapter two describes an overexpression system for PBP5. Overexpression of PBP5 in the periplasmic space causes lysis of the cells. By removing the DNA coding for the signal peptide a 200-fold cytoplasmic overexpression of PBP5 could be obtained. A new purification method for the purification of large quantities of both wild type and mutated forms of PBP5 was set up. Using the immobilized dye Procion rubine MX-B, PBP5 can be purified, independent of a functional active site, in a one-step procedure.

Sequence comparisons with other PBPs and β-lactamases reveal that PBP5 has an extra 100 residues at its C-terminus. The C-terminal 18 of these extra residues form a membrane anchor. Removal of this anchor results in a soluble, enzymatically active form of PBP5. In chapter three the domain structure of PBP5 was investigated by introducing stopcodons at various points in the sequence. A

shortened form of PBP5 missing 100 C-terminal amino acids still appeared to be enzymatically active, although it was more sensitive to proteolytic degradation.

PBPs and β-lactamases are members of a family of penicillin recognizing enzymes. A number of highly conserved amino acid motifs, all lining the active site, can be identified in all these enzymes. Chapter four describes mutation of the conserved residues using site-directed mutagenesis. The results emphasize the importance of these residues for the enzymatic activity of PBP5 and confirm their crucial role in catalysis.

Electron microscopy of thin sections revealed the formation of crystals in the cytoplasm of cells accumulating PBP5. The oblong crystals extended through the whole length of the cell, showing a substructure in both parallel and perpendicular cross-sections. Investigation of these crystals using image processing is described in chapter five.

In cooperation with the university of Liege *in vitro* crystallization experiments have been carried out. Diffraction patterns of several soluble forms of PBP5 are presented in chapter six.

Chapter seven describes a series of experiments concerning PBP6. Although PBP6 is described as having the same activity as PBP5, no D,D-carboxypeptidase activity could be determined for purified PBP6. Overexpression of PBP6 does not lead to lysis of the cells and therefore the *in vivo* function of PBP6 also seems to be different from PBP5. Since an elevated level of PBP6 has been observed in stationary-phase cells, this protein is probably involved in stabilization of peptidoglycan during stationary-phase growth of the cell.