Inhibition and dynamics of a β-lactamase

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Summary

The availability of antibiotics to treat infectious diseases has revolutionised healthcare. The current rise of antibiotic resistance, coupled with the recent void in antibiotic discoveries, threatens to undermine that progress. The work presented in this thesis aims to contribute to a greater understanding of resistance and its evolution, at the protein level. The model protein that was used for these studies is BlaC, the β-lactamase of *Mycobacterium tuberculosis*. This bacterium causes the highest yearly human death count of all pathogens and BlaC provides it with resistance to a broad spectrum of β-lactam antibiotics. The protein can be inhibited by β-lactamase inhibitors, enabling treatment of the disease with the combination of these inhibitors and β-lactam antibiotics. This treatment option is gaining popularity as a last-resort treatment option for tuberculosis bacteria that have gained resistance to other anti-tuberculosis drugs. Chapter 1 provides an introduction to and description of BlaC, as well as an introduction to some of the methodologies that were used here to study it.

Chapter 2 deepens the functional understanding of BlaC by specifying its activity in various conditions. The reversibility of BlaC inhibition by clavulanic acid is addressed especially. Whereas previous literature was inconsistent with regards to whether or not BlaC is able to hydrolyse clavulanic acid, we show conclusively that indeed it is. The hydrolysis is heavily dependent on the conditions. Specifically, the presence of phosphate, which binds to the active site with an affinity of *ca*. 30 mM, accelerates the hydrolysis of clavulanic acid by more than an order of magnitude. This finding adds to an existing volume of work on BlaC, which altogether provides a functional and structural understanding of the protein, which is, however, based on a static view of its structure. Proteins in solution at physiological temperatures do not exist in a single conformation but rather as a dynamic ensemble of conformations. Exchange between different conformations occurs on time scales ranging from picoseconds to second or even hours. The fast exchange is observed for processes on the low Ångström scale, for example, rotation of methyl groups, whereas larger motions, such as concerted movement of domains and unfolding are much slower. Some of these exchange processes and their time scales, magnitudes and localisation in the protein can be studied by nuclear magnetic resonance (NMR) spectroscopy. Chapter 3 provides the first such description of the dynamic behaviour of BlaC. It was found to be very rigid on fast time scales, showing very little local movement. On the millisecond time scale, however, significant motion was detected, specifically in the active site. These findings resemble those on related β-lactamases, and the rate of the active site motion was accurately determined for the first time, at *ca*. 860 s^{-1}. This chapter also provides the first insights into the effect of ligand binding on β-lactamase dynamics. It was found that

upon binding of inhibitor clavulanic acid, BlaC dynamics on all time scales increase dramatically. Fast motions in the α-domain of the enzyme indicate loss of stability of the hydrophobic core, while the direct observation of multiple conformations indicated either a shift in equilibrium and decrease in exchange rate of the active site motions, or the introduction of a second, very slow process.

As the use of β-lactam / β-lactamase inhibitor combinations to treat tuberculosis is gaining traction, it is also interesting to investigate if and how BlaC can potentially mutate to gain resistance to inhibition. Chapter 4 describes the development and application of a simulated evolution assay to identify mutations that confer resistance to clavulanic acid. The mutation that was found to confer most resistance, K234R, had already been described kinetically by Egesborg *et al.*, but nothing was yet known about the potential role of dynamics in the inhibitor-resistant phenotype. Chapter 4 therefore also describes NMR dynamics studies on this mutant and another mutant, G132N, which was identified by Soroka *et al.* to convey the same inhibitor-resistant phenotype upon BlaC. The results are surprisingly different. The G132N mutation causes an extensive region around the BlaC active site to experience two almost equally populated conformations, exchanging at *ca*. 70 s^{-1}. In the K234R mutant, however, no millisecond dynamics were detected at all. These results indicate that multiple evolutionary routes are available to reach the same inhibitor resistant phenotype. Furthermore, as the K234R mutant is active, they also show that active site dynamics on the millisecond time scale are not required for function.

Chapter 5 discusses the research presented in this thesis in the context of the overarching research goal of the Ubbink group and the overall progression of the scientific field. The work in this thesis adds to the understanding of BlaC inhibition and its dynamic behaviour, laying the foundation for detailed, ongoing studies of the roles of conserved residues and the laboratory evolution experiments aimed at understanding the evolutionary landscape of BlaC. Ultimately, we expect that this knowledge can help in the development of inhibitors that are less prone to resistance.