Abstract

β-lactams are one of the most frequently used antibacterial agents to date due to their selective toxicity. Unfortunately, the efficiency of these drugs is being challenged due to the production of several classes of β-lactamases. Both Gram-positive and Gram-negative bacteria use class A β-lactamases as the primary defence mechanism against the β-lactams to make them inactive. Apart from the three conserved motifs (SXXK, SXN, and KTG), the omega-loop present in the class A β-lactamases and a glutamic residue present in the loop plays a crucial role during β-lactam hydrolysis. Also, some of the penicillin-binding proteins (PBPs) with dual activity (DD-carboxypeptidase and β-lactamase) possess omega-loop. So, understanding the molecular mechanisms underlying the involvement of omega-loops in penicillin-interactive enzymes (PIEs) is vital for designing inhibitor molecules against it. With the above information in mind, in this work, we aimed to understand the role of the omega-loop on the structure-function relationship of two putative PIEs (MSMEG_1661 and MSMEG_4337) of mycobacteria based on *in silico*, *in vivo*, and *in vitro* analyses. In addition, we intended to design some peptide inhibitors specifically targeting the omega-loop of class A β-lactamases. Accordingly, the constructs of the genes of both membrane-bound and soluble forms of proteins were created in pBAD18-cam and pET28a(+) vectors, respectively, and expressed. Expressed soluble proteins were purified and subjected to *in vitro* experimental studies. Interestingly, *in vivo* expressions of MSMEG_1661 and MSMEG_4337 were able to restore the normal rod-shaped morphology of the aberrantly-shaped *E. coli* septuple PBP deletion mutant. The result resembled the *in vivo* morphology maintenance activity of DD-CPase enzymes. Also, MSMEG_1661 demonstrated β-lactamase activity in Δ*ampC* mutant of *E. coli* showing β-lactam resistance, whereas MSMEG_4337 indicated *in vivo* DD-CPase activity (resistance towards SK2O56(*ΔPBP5ΔPBP6). In silico* investigations revealed that the omega-loop supposedly plays a significant role in exerting the activities of these two enzymes. Secondly, to validate our hypothesis that masking of glutamic acid in the omega-loop of class A β-lactamase might affect the β-lactam sensitivity, we have designed several pentapeptides covering the short stretch of amino-acids around the glutamic acid in the omega-loop. We implemented various *in silico* screening protocols (peptide modelling, docking, and MD simulation) to find out 6 suitable peptides and synthesized them manually using the Fmoc Solid-Phase Synthesis Peptide (SPPS) and further purified them by HPLC. The hydrolysing capacity of some specific known β-lactamases, TEM-1 and SHV-14, were tested (both *in-vivo* & *in vitro*) against a selection of the β-lactam drugs in the presence and absence of the synthesized peptides. To our surprise, upon *trans* expression of β-lactamase, there was a significant impact of the peptides on the resistance profile of some of the bacteria, and the antimicrobial activity of β-lactam drugs was enhanced. Additionally, a systematic virtual screening approach was followed to propose novel cephalosporin-based molecules as a potent inhibitor of class A β lactamase. Using virtual screening, ADMET analysis, and molecular dynamics studies, three possible lead compounds were chosen based on their binding affinity and interaction profile with the omega-loop. Overall, we infer that suppression of β-lactamase activity can be achieved by designing inhibitors against the omega-loop of β-lactamases.

Keywords: β-lactams, β-lactamases, PBPs, DD-CPases, Antimicrobial peptides, Virtual screening, ADMET analysis, MD simulation