Abstract

Penicillin-binding proteins (PBPs), membrane-anchored ectoproteins, are instrumental in the polymerization and remodelling of the peptidoglycans (PG), the stress-bearing component of the bacterial cell wall. PBPs have been exploited as the targets of the highly successful antibiotics, β-lactams. PBP5, the most abundant low molecular mass (LMM) PBP, functions as a D-alanine carboxypeptidase (DD-CPase) that cleaves the C-terminal D-alanine from cell wall pentapeptides. DacD, a member of LMM-PBPs, has sequences similarities with *E. coli* PBP5. However, the function(s) of DacD in *E. coli* or its homologue in *K. pneumoniae* (KDacD) is unclear. In addition, the function of AmpH, another LMM-PBP that has been claimed to possess DD-CPase activity remains unexplored. To assess the nature of the DacD, KDacD and AmpH, the genes of the soluble form of these enzymes (excluding signal peptide and membrane anchors), were cloned, *viz.*, sDacD, sKDacD and sAmpH, respectively. The proteins were overexpressed in *E. coli*, purified through ampicillin-affinity chromatography and subsequently analyzed for their kinetic behaviours. The sDacD exhibited a comparatively higher DD-CPase activity than sKDacD and sAmpH *in vitro* against peptidoglycan mimetic pentapeptide substrate. AmpH assists biofilm formation in *E. coli*. To address the molecular interactions, *in silico* 3D-models of the proteins were created and their structure-function relationships were studied with reference to the *E. coli* PBP5. The *in silico* analyses hypothesize active site grove volume of DacD might act as a determinant of its DD-CPase activity. Among all the LMM-PBPs, PBP5 is known for its high DD-CPase activity. However, structurally it shares similarity with the class A β-lactamases (TEM-1) around their penicillin-binding domain. PBP5 possesses an equivalent Ω-like loop of β-lactamases. To understand the physiological significance of the 'Ω-like' loop in PBP5, A184E is generated (PBP5_A184E). The mutant protein was able to revert back the deformed cell shape of CS703-1 (seven PBP deletion mutant of CS109), thus maintains the consistency with its unchanged DD-CPase activity. Surprisingly, PBP5_A184E shows a dramatic increase in β-lactam resistance towards both penicillin and cephalosporin group of antibiotics as compared to the cells expressing PBP5. Therefore, the point mutation introduces β-lactamase nature in PBP5 without affecting its DD-CPase activity.