Abstract

Penicillin-binding proteins (PBPs), targets of beta-lactam antibiotics, are the cytoplasmic membrane-bound enzymes involved in the peptidoglycan crosslinking and remodelling. Most abundant among the PBPs is PBP5, which is a DD-Carboxypeptidase (DD-CPase) that helps maintain cell shape and intrinsic beta-lactam resistance in *E. coli*. However, little is known about its homologues in *Klebsiella pneumoniae*. To assess the biochemical nature of the DD-CPase homologues DacA and DacC of *K. pneumoniae*, the genes of the soluble form of these enzymes (i.e., devoid of signal peptide and membrane anchors) *viz.*, sKDacA and sKDacC, respectively were cloned, overexpressed in *E. coli*, purified through ampicillin-affinity chromatography and analyzed for their kinetic behaviours. The sKDacC exhibited better DD-CPase activity than sKDacA *in vitro* against peptidoglycan mimetic pentapeptide substrate. To understand the molecular nature, *in silico* 3D-models of the proteins were created and analyzed by comparing them with *E. coli* PBP5, which revealed smaller active site groove volume than *E. coli* PBP5. However, both sKDacA and sKDacC possess 'Ω-like loop' similar to the one present in *E. coli* PBP5. In general, 'Ω-loop' is a region of class A beta-lactamase in which Glu166 residue helps in deacylation of beta-lactams. Therefore, to understand the physiological significance of the 'Ω-like' loop in DD-CPase, Leu182 residue in 'Ω-like' loop of *E. coli* PBP5 was mutated to glutamic acid (creating PBP5L182E). PBP5L182E had inefficient DD-CPase activity as revealed by *in vitro*, *in vivo* and *in silico* analyses. Therefore, Leu182 of *E. coli* PBP5 appeared as a determinant of its DD-CPase activity. Similarly, the corresponding leucine residue of 'Ω-like loop' of KDacC was replaced with glutamic acid and analyzed its activity. Unlike *E. coli* PBP5, upon expression of KDacC in deformed shaped PBP deletion mutant of *E. coli*, the cells were swelled while its expression in the wild type *E. coli* or *K. pneumoniae* generated pear-shaped phenotype, though no change was observed upon expressing KDacCL203E. In addition, *in trans* expression of KDacC made the *E. coli* PBP5 deletion mutant sensitive towards beta-lactam, but the sensitivity was unaltered upon expression of KDacCL203E. Further, the soluble form of KDacCL203E (smKDacC) exhibited lower DD-CPase activity. Overall study indicates that the leucine residue of 'Ω-like loop' that faces the active-site of DD-CPase is important for the enzymatic activity, both in *E. coli* and *K. pneumoniae*.