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Structural insight of NDM-1: Understanding functional state of protein

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New Delhi metallo-β-lactamase-1 (NDM-1) is expressed by various members of Enterobacteriaceae as a defense mechanism to hydrolyze β-lactam antibiotics. Despite various studies showing the significance of active site residues in the catalytic mechanism, there is paucity of reports addressing the role of non-active site residues in the structure and function of NDM-1. Here, we investigated the significance of non-active site residue Trp-93 in the structure and function of NDM-1. We cloned bla_{NDM-1} from *E. cloacae* clinical strain (EC-15) and introduced Trp93Ala mutation by PCR based site-directed mutagenesis. Proteins were expressed and purified to homogeneity by affinity chromatography. The MICs of Trp93Ala mutant were reduced by 4-8 folds against ampicillin, cefotaxime, ceftazidime, cefoxitin, imipenem and meropenem. The poor hydrolytic activity of Trp93Ala mutant was also reflected by its reduced catalytic efficiency. The overall catalytic efficiency of Trp93Ala was reduced by 40-55% (K_m was reduced while k_{cat} was similar to that of wtNDM-1). Heat-induced denaturation showed that ΔG_D^o and T_m of Trp93Ala mutant was reduced by 1.8 kcal/mol and 4.8 °C, respectively. Far-UV CD analysis showed that the α-helical content of Trp93Ala mutant was reduced by 2.9 %. The decrease in stability and catalytic efficiency of Trp93Ala mutant was due to the loss of two hydrogen bonds with Ser-63 and Val-73 and hydrophobic interactions with Leu-65, Val-73, Gln-123 and Asp-124. The study provides an insight into the role of non-active site amino acid residues in the hydrolytic mechanism of NDM-1.

Biography

Asad U Khan is a Molecular Biologist, completed his graduation from Biochemistry Department, Aligarh Muslim University, India and Post-Doctorate from RUTGERS, USA. Currently he is a Professor in the Interdisciplinary Biotechnology Unit, AMU, India. He is a Member of Editorial Board of a number of international journals and has over 169 research articles to his credit. He is also a Member of several international grant review committees and elected Member of Royal Society of Chemistry.

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