Poster Presentation

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Structural basis for the evolution of vancomycin resistance D,D-peptidases

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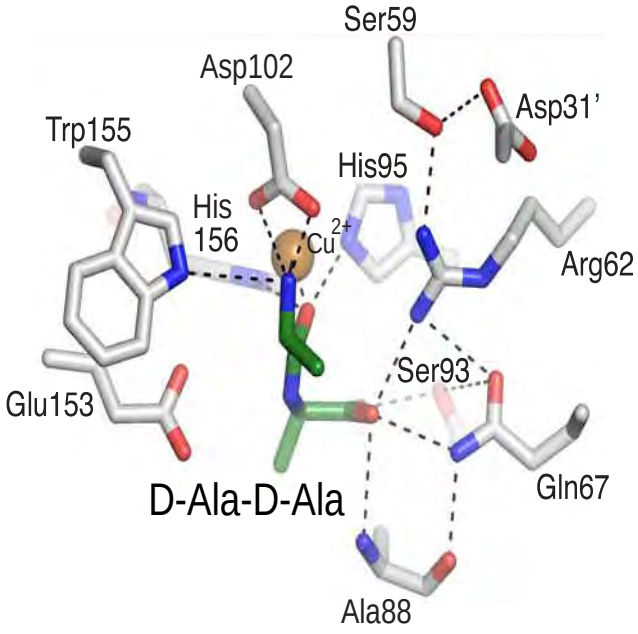
Emergence of high-level resistance to the last resort glycopeptide antibiotic vancomycin in Enterococcus spp. and its spread to methicillin-resistant Staphylococcus aureus is a public health threat. Resistance to vancomycin is due to substitution of the D-Ala-D-Ala terminus of cell wall precursors, which forms the antibiotic target, by D-Ala-D-Lac or D-Ala-D-Ser of low binding affinities. Resistance also requires depletion of the normal precursors catalyzed by the zinc-dependent D,D-peptidases VanX and VanY acting on dipeptide (D-Ala-D-Ala) or pentapeptide (UDP-MurNac-L-Ala-D-γ-Glu-L-Lys-D-Ala-D-Ala), respectively. Some resistance operons encode VanXY D,D-peptidase acting on both substrates. Van D,D-peptidases represent attractive targets for combinational antimicrobial therapies to curb resistance; however, the molecular basis of their specificity remains poorly understood, hindering development of potent inhibitors. Therefore we undertook detailed structure-function analysis of VanXY and VanY enzymes. Obtained structural information revealed the substrate-binding site of VanXYC as an extended cavity suitable for binding of di- or pentapeptides, contrasting with previous results showing that VanX contains a small, shallow active site. Biochemical and mutagenesis analysis identified a mobile cap over the catalytic site of VanXYC as the key structural element involved in a switch between di- and pentapeptide hydrolysis. The structures also provided the molecular basis for selectivity towards Van-susceptible peptidoglycan precursors. Overall, this study illustrates the adaptability of the D,D-peptidase fold in response to antibiotic pressure via evolution of particular structural elements that modulate substrate specificity. The results open new opportunities for structure-guided development of Van D,D-peptidases specific inhibitors as glycopeptides adjuvants. This project has been funded by NIAID under Contracts No. HHSN272200700058C and HHSN272201200026C.

[1] *P. Stogios, D. Meziane-Cherif, PNAS, 2014, 111(16), 5872-7*



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