Invited Lecture

Bacterial Cell-wall Recycling and the Link with Antimicrobial Resistance

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The spread of antimicrobial resistance (AMR) might be one of the world's health biggest problems, jeopardizing the treatment of infections worldwide. Alarming levels of drug resistance have been reported worldwide, with the result that common infectious diseases are becoming untreatable. The bacterial cell wall is an essential gigantic macromolecule that defines the shape of the bacterium and enables the bacterium to resist lysis as a result of its high intracellular osmotic pressure. The main component of cell wall is peptidoglycan (PG) that consists of repeating linear polymers of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) linked together via short oligopeptide chains. Because the cell wall is structurally specific of bacteria, the steps involved in regulation of cell-wall biosynthesis are the targets of numerous antibiotics, including the -lactams that represent >50% of the available contemporary antibiotic arsenal. The balance between the integrity of the bacterial cell wall and the activation of resistance mechanisms to deactivate antibiotics directed at the cell wall implies, on the one hand, the orchestration between the synthesis and remodeling mechanisms of the cell wall, and on the other hand, the detection and response to antibiotics by modulating genetic regulation by specific effectors.

In multidrug-resistant pathogen *Pseudomonas aeruginosa*, PG remodeling produces cell-wall fragments that are recycled but can also act as messengers for bacterial communication, as effector molecules in immune response and as signaling molecules triggering antibiotic resistance. The enzymatic processes that build, remodel, and recycle the chemical components of this cross-linked polymer are preeminent targets of antibiotics and exploratory targets for emerging antibiotic structures. We recently reported a comprehensive kinetic and structural analysis for one such enzyme, the *P. aeruginosa* anhydro-*N*-acetylmuramic acid (anhNAM) kinase (AnmK). Computational simulations in conjunction with the high-resolution X-ray structures revealed the full catalytic cycle. We further reported that a *P. aeruginosa* strain with disrupted *anmK* gene is more susceptible to the β-lactam imipenem compared to the WT strain [1]. These observations position AnmK for understanding the nexus among peptidoglycan recycling, susceptibility to antibiotics, and bacterial virulence. Under the -lactam challenge *P. aeruginosa* attempts to repair the aberrantly formed peptidoglycan by the function of the lytic transglycosylase Slt. On the other hand, natural product bulgecin A potentiates the activity of β-lactam antibiotics by inhibition of the lytic transglycosylases Slt, MltD and MltG. We have shown how Slt performs the repair activities on cell wall and how this protein is inhibited by bulgecin A [2]. Besides, we recently reported the 3D structures of MltD in complex will bulgecin A and PG analogues [3], providing evidences on the inhibition mechanism and on the MltD activity in vivo.

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[2] Lee, M., Batuecas, M. T., Tomoshige, S., Domínguez-Gil, T., Mahasenan, K. V., Dik, D. A., Hesek, D., Millán, C., Usón, I., Lastochkin, E., Hermoso, J. A. & Mobashery, S. (2018) *Proc. Natl. Acad. Sci. USA* **115**(17), 4393.

[3] Miguel-Ruano, V., Feltzer R., Batuecas, M.T., Ramachandran, B., El-Araby, A.M., Avila-Cobian, L.F., De Benedetti S., Mobashery, S. & Hermoso, J.A. (2024) *Int. J. Biol. Macromol.* **267**, 131420.