Inhibition of *Pseudomonas aeruginosa* biofilms: new molecular strategies targeting cyclic-di-GMP metabolism

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Biofilms formed by bacterial pathogens are responsible of more than 70% of all infections in developed countries and are less sensitive to treatments with antimicrobial agents. The ubiquitous second messenger 3', 5'-cyclic di guanylic acid (c-di-GMP) is used in most bacteria to control the switch to the biofilm lifestyle; c-di-GMP has no counterpart in eukaryotic cells and thus it is an ideal target to develop effective anti-biofilm strategies.

The aim of this proposal is to study the metabolism of c-di-GMP in the opportunistic human pathogen *Pseudomonas aeruginosa* in order to find new targets for effective anti-biofilm drugs. We plan to characterize strategic *P. aeruginosa* proteins involved in c-di-GMP metabolism (diguanylate cyclases-DGCs and phosphodiesterases-PDEs) and to study the connection between the c-diGMP pathway and other metabolic pathways relevant for pathogenesis.

We developed the enzymatic and c-di-GMP binding assays using the reference DGC PleD from *Caulobacter crescentus*. We have measured the DGC activity of purified PleD and confirmed, by titration of PleD with c-di-GMP (employing Isothermal Titration Calorimetry-ITC), that PleD binds c-di-GMP (2 mol c-di-GMP/mol protein; Kd 0,9 µM) in agreement with literature data. We have tested putative DGC inhibitors, starting from selected compounds, previously identified in microbiological screening of a chemical library by Landini and coworkers. These compounds do not significantly inhibit PleD *in vitro* (up to 500 µM) suggesting that they do not inhibit c-di-GMP biosynthesis through direct interaction with DGC proteins, but likely affect the availability of nucleotide substrate(s).

We have characterized putative *P. aeruginosa* DGCs or PDEs, focusing our attention on selected targets, i.e. the DGC PA1120 and the PDEs PA4781 and PA4108. The PA1120 protein (TpbB) is a DGC linking Las-induced quorum sensing (QS) to the formation of matrix exopolysaccharide (EPS) and extracellular DNA, necessary for biofilm development and coordinated group response in *P. aeruginosa*. We have produced the isolated catalytic domain of TpbB and measured its catalytic activity (0,1 nmol/min/mg); PA1120 is not significantly inhibited by c-di-GMP at low GTP concentrations. Identification and characterization of inhibitors are in progress.

Few biochemical data are available on PDEs containing the HD-GYP domain, despite their importance in pathogenesis and their role in controlling biofilm formation. While EAL-type PDEs hydrolyze c-di-GMP to linear diguanylate (pGpG), HD-GYPs completely hydrolyze c-di-GMP to GMP in a single reaction. In *P. aeruginosa*, 2 HD-GYP proteins are found, which are able to decrease c-di-GMP levels *in vivo*; these proteins contain the PDE catalytic
domain and a (putative) regulatory domain, either of the CheY-type (PA4781) or unknown (PA4108). Both proteins were purified and tested for their PDE activity \textit{in vitro}, monitoring c-di-GMP hydrolysis to GMP: while the PA4108 enzyme shows c-di-GMP hydrolytic activity, PA4781 is inactive. In order to bypass a possible activation step, a truncated version of PA4781 was also analyzed (PA4781-G) and found to be inactive \textit{in vitro}, but active in cell extracts or in intact \textit{E. coli} cells. These results suggest that the activity of PA4781 may depend on the interaction with an intracellular partner, as previously proposed for other HD-GYP PDEs.

In parallel, we have also studied Aromatic L-Aminoacid decarboxylase (DOPA decarboxylase-DDC), the human enzyme responsible for a key step in the synthesis of adrenaline, one of the eukaryotic signal molecules sensed by Gram-negative pathogens. These molecules bind to bacterial membrane receptors and activate signaling pathways involving, among others, c-diGMP, thus leading to modulation of biofilm formation. Analysis of the three-dimensional structure coupled to a kinetic study allows to identify the structural determinants of the open/close conformational change occurring upon PLP binding and thereby propose a model for the stability of Group II decarboxylases \textit{in vivo} (Giardina \textit{et al.}, 2011).

**Publications**


**Research Group**

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**Collaborations**

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