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Poster presentation

Quantitation of cyclic dinucleotides by reversed-phase LC-MS/MS

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Cyclic nucleotides function as second messengers in all kingdoms of life. Recently, cyclic dinucleotides (c-di-NMPs) have been identified as bacterial signaling molecules. Cyclic diguanosine monophosphate (c-di-GMP) plays a major role in the modulation of bacterial surface components. Synthesis and degradation of c-di-GMP is controlled by so-called GGDEF (diguanylate cyclase activity) and EAL (phosphodiesterase activity) domains, respectively. These domains influence the sessility and motility of bacteria *via* regulation of biosynthesis of exopolysaccharides (e.g. cellulose) and fimbriae. Thus, the c-di-GMP level affects the formation of biofilms that represent the predominant form in which bacteria exist in the natural environment. The progress and persistence of many infectious diseases is based on biofilm formation by bacterial pathogens.

Hence, there is a great need for the establishment of a robust and sensitive quantitation method for cyclic dinucleotides. Recently, a matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF) method for the detection of c-di-GMP has been reported [1]. However, quantitation of low molecular weight molecules by MALDI-TOF is rather problematic and the described method requires a tedious separate chromatographic work-up step prior to the sample preparation for the final MALDI-TOF analysis. We have established a much more simple and sensitive quantitation method for c-di-NMPs using a reversed-phase liquid chromatography-coupled tandem mass spectrometry (LC-MS/MS) system with xanthosine 3',5'-cyclic monophosphate (cXMP) as internal standard (LOD = 4 ng/mL, see Figure 1). Cyclic diadenosine monophosphate (c-di-AMP) would be the most suitable internal standard in terms of close structural relation and comparable chromatographic behavior. However, c-di-AMP may also be present in bacteria and thus might interfere with the determination of c-di-GMP levels.

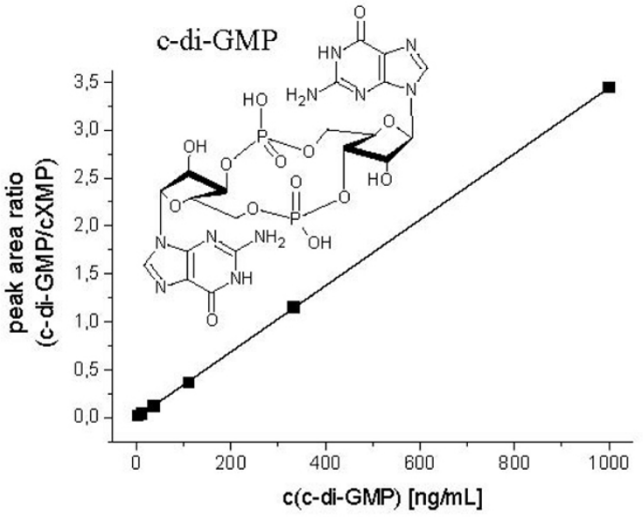

Figure 1
Calibration curve for c-di-GMP obtained by LC-MS/MS with a reversed-phase column and cXMP as internal standard.

We will apply the established method for the quantitation of c-di-NMPs in various *Pseudomonas aeruginosa* strains and aim at determining the enzymatic activity of diguanylate cyclase.

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References

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