Institute of Pharmacology

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Viral phosphodiesterases circumvent bacterial phage defense – A characterization of the cyclic nucleotide-cleaving PDE Apyc1

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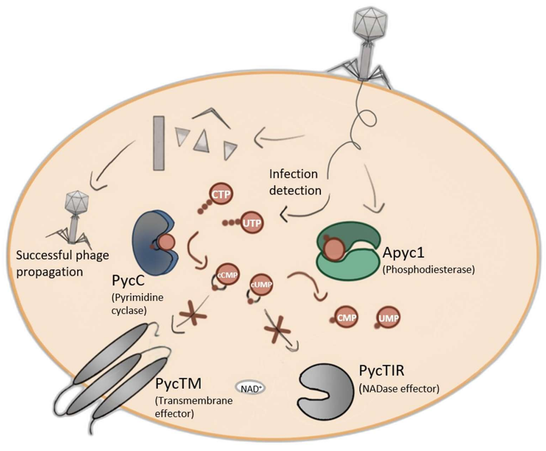
Introduction

Multi-drug-resistant bacteria pose a growing threat to the health of the population. Therefore, new antibacterial drugs are urgently needed [1]. This work deals with the characterization of a novel viral phosphodiesterase (Apyc1) as a potential antibacterial drug candidate. In nature, Apyc1 enzymes play a key role in the infection of bacteria by phages. To defend themselves, bacteria have developed various anti-phage defense systems. The system Pycsar relies on the activation of host immunity by formation of pyrimidine cyclic nucleotides (3',5'-cCMP and 3',5'-cUMP) [2]. Due to evolutionary pressure, phages have developed mechanisms to circumvent bacterial immunity. Phage phosphodiesterases (PDEs) with a relaxed cyclic nucleotide specificity play a central role in disrupting the host immunity of bacteria. These PDEs are able to prevent the activation of Pycsar by cleaving 3',5'-cUMP and 3',5'-cCMP and are therefore referred to as anti-Pycsar 1 enzymes (Apyc1) [3]. This work aims to test whether Apyc1, in contrast to other PDEs, can also hydrolyze 2',3'-cNMPs, whose role as signaling molecules in bacteria is still not fully understood.



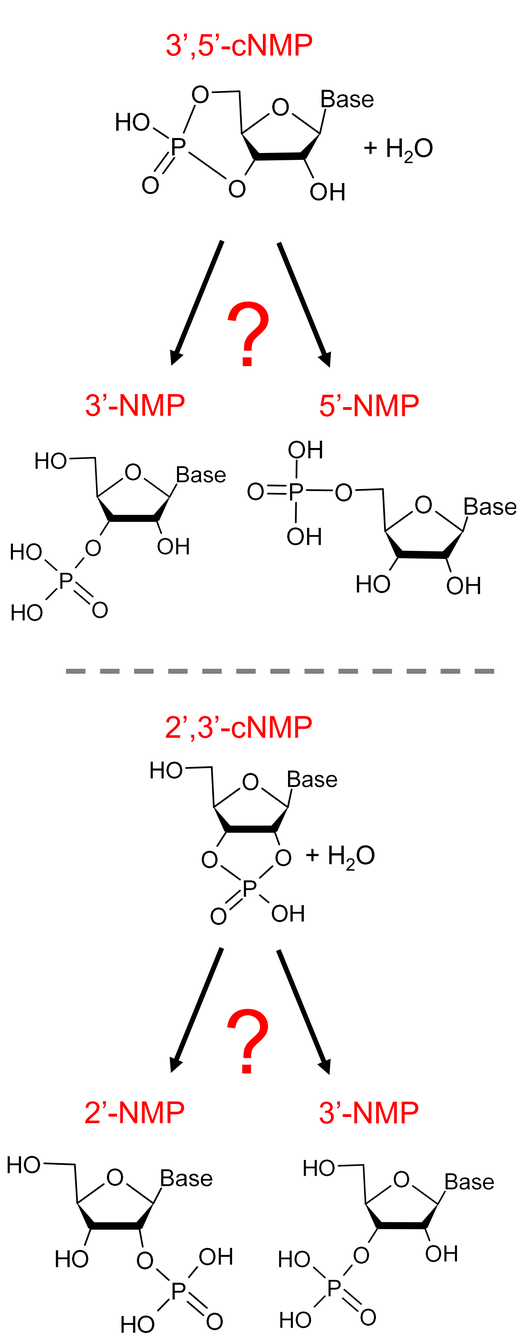
Anti-Pycsar scheme based on Tal et al. [2].

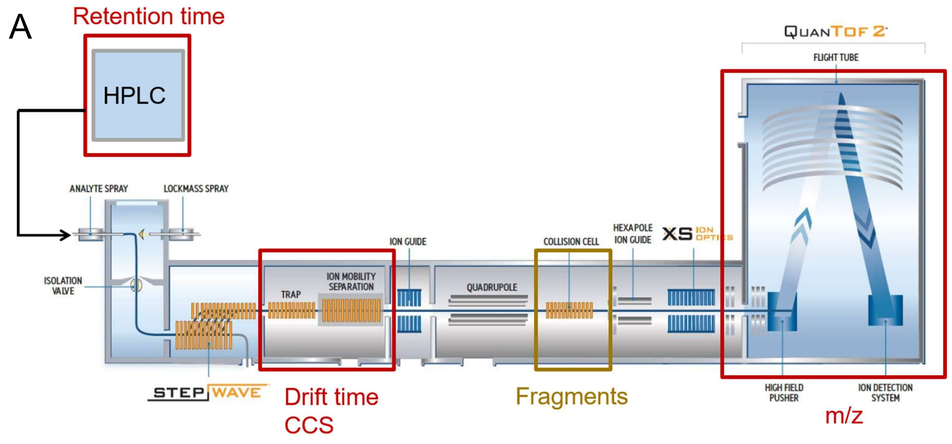
Methods

Apyc1 gene from *Bacillus* phage SBSphiJ was cloned into a pET expression vector containing an N-terminal 6x His-tag. After production in *E. coli* BL21(DE3)pLysS, Apyc1 was purified via immobilized metal affinity chromatography and size exclusion chromatography. SDS-PAGE and western blot were used for identification and purity determination. The purified enzyme was then analyzed in enzyme assays containing 3',5'-cNMPs and 2',3'-cNMPs as substrates. Tris-HCl buffer (50 mM, pH 7.5) containing 150 mM NaCl, 1 mM DTT, 5 mM $MgCl_2$, 1 mM $MnCl_2$ and 100 µM of each 3',5'- or 2',3'-cNMP was used as a reaction buffer. The products of these reactions were unequivocally identified via LC-IMS-QTOF.

Methodology and Results

Identification of 3',5'-cNMP and 2',3'-cNMP hydrolysis products





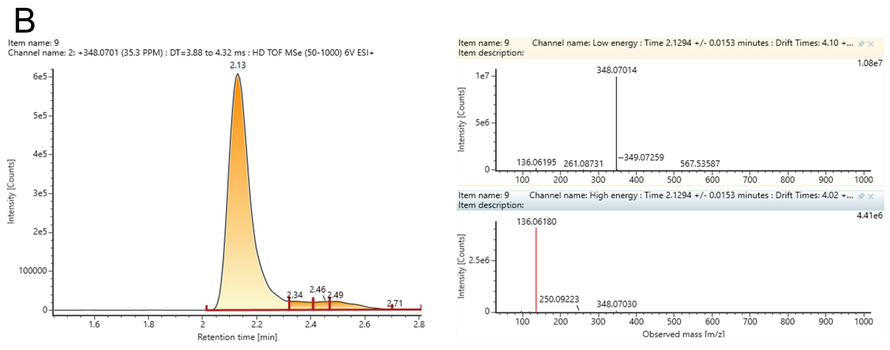


Figure 1: (**A**) Schematic diagram of the Vion LC-IMS-QTOF platform used in this work [4]. (**B**) Exemplary mass spectrum of product 1 of the 3',5'-cNMP hydrolysis identified as 5'-AMP.

Table 1: Comparison of LC-IMS-QTOF measurements of 3',5'-cAMP, -cCMP, -cGMP, -cUMP and 2',3'-cAMP, -cCMP, -cGMP, -cUMP hydrolysis products to NMP standard substances.

	3',5'-cNMP hydrolysis				NMP standards							
					AMP		CMP		GMP		UMP	
NMP	**Product 1**	**Product 2**	**Product 3**	**Product 4**	**3'**	**5'**	**3'**	**5'**	**3'**	**5'**	**3'**	**5'**
m/z	348.0702	324.0588	364.0650	325.0430	348.0700	348.0700	324.0590	324.0587	364.0647	364.0643	325.0420	325.0420
RT [min]	2.13	1.56	2.36	1.71	3.03	2.14	2.00	1.56	3.55	2.37	2.45	1.75
Drift time [ms]	4.10	3.97	4.50	3.90	4.24	4.07	4.02	3.93	4.61	4.45	3.96	3.88
CCS [Å²]	168.81	165.83	178.13	164.31	171.38	167.23	166.21	164.10	180.06	176.24	164.85	162.95

	2',3'-cNMP hydrolysis				NMP standards							
					AMP		CMP		GMP		UMP	
NMP	**Product 1**	**Product 2**	**Product 3**	**Product 4**	**2'**	**3'**	**2'**	**3'**	**2'**	**3'**	**2'**	**3'**
m/z	348.0700	324.0589	364.0646	325.0427	348.0700	348.0670	324.0581	324.0590	364.0645	364.0647	325.0400	325.0420
RT [min]	3.06	2.02	3.60	2.44	4.93	3.03	2.69	2.00	5.45	3.55	2.70	2.45
Drift time [ms]	4.24	4.05	4.59	4.00	4.17	4.24	3.96	4.02	4.38	4.61	3.90	3.96
CCS [Å²]	171.92	167.63	180.33	166.62	169.63	171.38	164.96	166.21	174.64	180.06	163.47	164.85

- Regiospecific hydrolysis: 3',5'-cNMPs exclusively yielded 5'-NMPs; 2',3'-cNMPs exclusively yielded 3'-NMPs.
- Different nucleotides were distinguished by comparing experimental m/z values of the hydrolysis products with those of NMP standards.
- Nucleotide isomers were identified through comparison of retention time, drift time and CCS values against 2'-,3'-, and 5'-NMP standards.

Conclusion and Outlook:

- Apyc1-SBSPhiJ exhibits hydrolytic activity toward both 3',5'-cNMPs and 2',3'-cNMPs.
- The hydrolysis products of 3',5'-cNMPs and 2',3'-cNMPs were identified as the corresponding 5'-NMPs and 3'-NMPs, respectively.
- Future work will focus on optimizing the enzyme assay conditions, followed by a quantitative analysis of Apyc1 activity.
  - Determination of kinetic parameters, identification of modulators of Apyc1 activity

Poster PDF

References

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