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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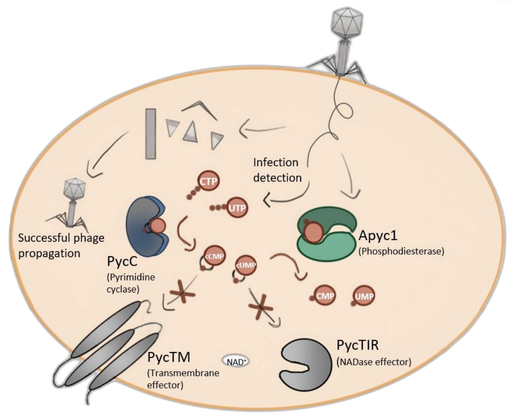
Introduction

Bacteria have developed various anti-phage defense systems to defend against phage infection. The defense system "Pycsar" relies on the activation of host immunity by formation of pyrimidine cyclic nucleotides (3',5'-cCMP and 3',5'-cUMP) [1].
Due to evolutionary pressure, phages, on the other hand, 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) [2]. The aim of this work is to characterize Apyc1-PDEs, as they may be candidates for novel classes of antibacterial or antiviral drugs [3].



Scheme based on Hobbs et al. [2].

Methods

Apyc1 genes from *Bacillus* phages SBSphiJ and Bsp38 were cloned into a pET expression vector containing an N-terminal 6x His-tag. After production in *E. coli* BL21(DE3)pLysS, Apyc1 enzymes were purified via immobilized metal affinity chromatography and size exclusion chromatography. SDS-PAGE and western blot were used for identification and purity determination of Apyc1-SBSphiJ and Apyc1-Bsp38. Different Apyc1 concentrations (1, 2.5, 5, 10 and 100 nM) were used for enzyme assays. Tris-HCl buffer (50 mM, pH 7.5) was used as a reaction buffer and contained 150 mM NaCl, 1 mM DTT, 5 mM $MgCl_2$, 1 mM $MnCl_2$ and 100 µM of each 3',5'-cNMP. The conversion of 3',5'-cNMPs to the corresponding 5'-NMPs was quantified after 30 min via LC-MS/MS.

Results

Alignment of Apyc1-Bsp38 and Apyc1-SBSphiJ protein sequences

Zn^{2+} coordinating — loop extending into cNMP binding pocket

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Bsp38     1   MLHTTQIRMVGTGSAFSKKFYNNSALVTFTNGYNLLIDCGHSVPKGLHDADIPLESIDGILITHTHADHIGGLEEVALYNKFVLGGRKIDLLVPNTLVESLWENSLKGGLRYSDT-YDDLSLSDYFTVRSLKTFTSGA  137
SBSphiJ   1   MAHTTQLTMVGTGSAFSKKFYNNSALVQFTNGYNLLIDCGHSVPKGLHDLGIPLESIDGILITHTHADHIGGLEEVALYNKFVLGGRKIDLLVPETLVEPLWENSLKGGLRYPDEDSPEPELSDYFTVRSLKTSDYGV  138

Bsp38     138 ARTQLEENIAIKLYPTFHVSHMASYAVGLEDRGEDKVFYSSDTIFDEYLIDYALTYSWVFHDCQFFTGGVHASLDELLNYIPEEDQDRVFLMHYGDNMEDFFTKTGRMRFALQGRTYIL*  257
SBSphiJ   139 AHTQIEENMAVRLYPTVHVSHMDSYAVGLVDRGEDKVFYSSDTIFDEYLIDYALTYPWVFHDCQFFTGGVHASLDELLNYIPEEDQARVFLMHYGDNLEDFFNKTGRMRFALQGRTYIL*  258
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Protein sequences were aligned using protein BLAST®. Identification of functional regions by Hobbs *et al.* [2].

Determination of purity and identity of Apyc1 homologs

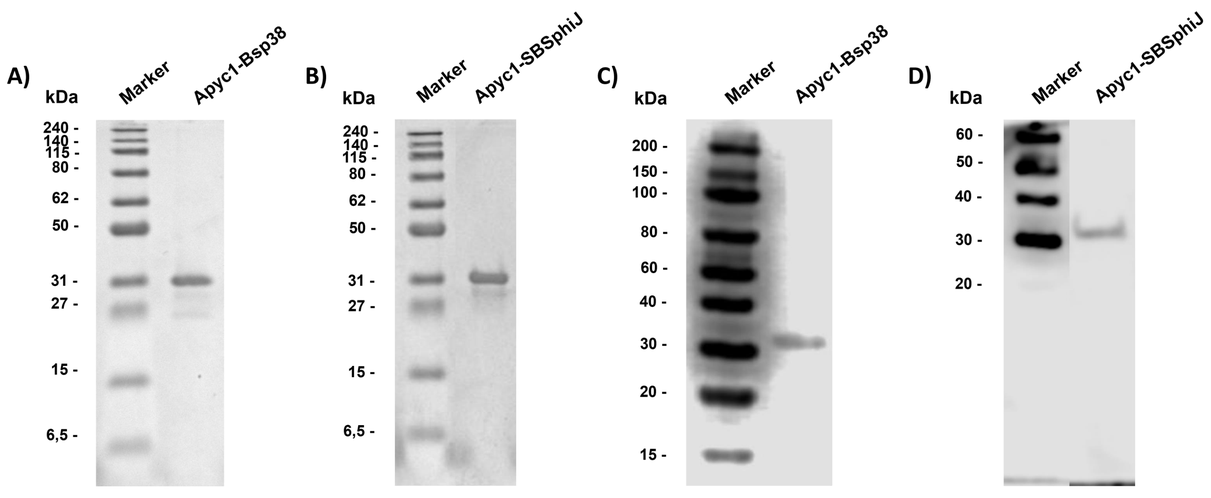

Fig. 1: SDS-PAGE analysis (**A and B**) of Apyc1-Bsp38 and -SBSphiJ after affinity and size exclusion chromatography. Staining was performed using a coomassie R-250 dye-based reagent.
Western blot analysis (**C and D**) of Apyc1-Bsp38 and -SBSphiJ after affinity and size exclusion chromatography. An anti-His-tag primary antibody and an HRP-conjugated anti-mouse secondary antibody were used for the immunoblot. Visualization of the secondary antibody was performed through via chemiluminescence.

- SDS-PAGE: Detected bands fit the size of the fusion proteins (Bsp38: 32.71 kDa, SBSphiJ: 32.74 kDa).
- Western blot: His-tags of the fusion proteins were successfully detected.
- Enzyme purities were determined by image evaluation (ImageJ).
  - Purity: 90.1 % (Bsp38) and 88.4 % (SBSphiJ)

Enzyme assay

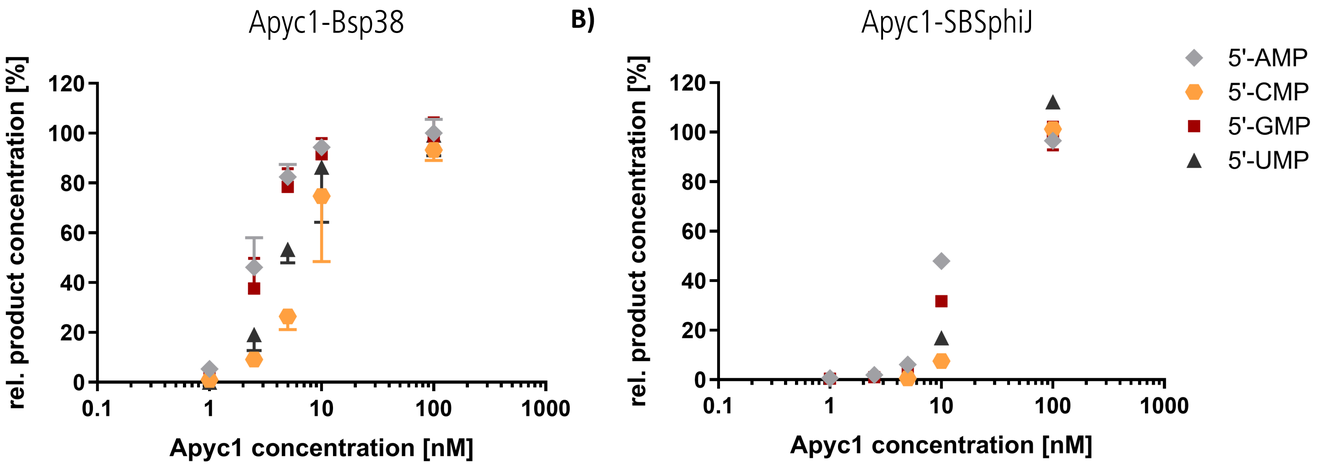

Fig. 2: Enzyme assays with purified Apyc1-Bsp38 (**A**) and Apyc1-SBSphiJ (**B**) in triplicates. Relative product concentrations (5'-AMP, 5'-GMP, 5'-UMP, and 5'-CMP) are presented as a function of enzyme concentration (1 nM, 2.5 nM, 5 nM, 10 nM, and 100 nM).

- Relative product concentrations rose with increasing enzyme concentration.
- Both Apyc1 homologs seem to cleave purine cyclic nucleotides faster than pyrimidine cyclic nucleotides.
  - 3',5'-cAMP and 3',5'-cGMP > 3',5'-cUMP and 3',5'-cCMP

Conclusion and Outlook:

- Apyc1 from *Bacillus* phage Bsp38 and SBSphiJ were successfully produced and purified.
- The activity of the enzymes was confirmed by enzyme assays. (Assumption: Faster conversion of 3',5'-cAMP and 3',5'-cGMP compared to 3',5'-cCMP and 3',5'-cUMP)
- In future work, temperature and pH optima of Apyc1 homologs will be determined.
- For a kinetic characterization of Apyc1 activity, the conversion of each substrate needs to be investigated separately.

References
[1] Tal *et al.* (2021). *Cell*, *184*(23), 5728-5739.e16.
[2] Hobbs *et al.* (2022). *Nature*, *605*(7910), 522–526.
[3] Seifert and Bugert (2023). *Trends Biochem Sci*, *48*(10), 835–838.

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Metabolomics

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