Production and characterization of recombinant VaaMPIII 3, a disintegrin like/cysteine rich protein from *Vipera a. ammodytes* venom

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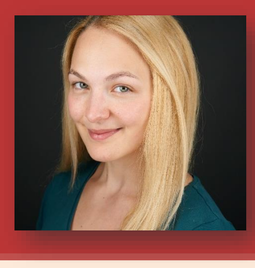
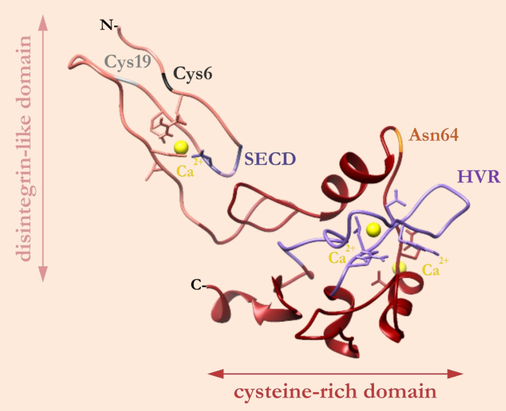


INTRODUCTION

VaaMPIII-3 is a disintegrin-like/cysteine-rich protein discovered in the venom of *Vipera a. ammodytes* (*Vaa*) that defines the new P-IIIe subclass of snake venom metalloproteinases[1]. It contains 17 Cys residues, one of which is free. Six isoforms of this 21 kDa monomeric glycoprotein were found in *Vaa* venom, with isoelectric points (pIs) ranging from 4.5 to 5.1. VaaMPIII-3 inhibits platelet aggregation induced by ADP, collagen or arachidonic acid, thus contributing to the anticoagulant effect of the venom. As such, it is an interesting molecule for the development of new antithrombotic drugs. The underlying mechanism of its action remains to be elucidated.

Natural VaaMPIII-3



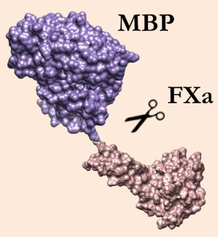
Legend	
Cys6	free Cys
Cys19	alternative free Cys (in 1 of 10 molecules)
Ca^{2+}	calcium ions
SECD	integrin-binding motif
Asn64	N-glycosylation site
HVR	hypervariable region

AIM

Recombinant production of VaaMPIII-3 (rVaaMPIII-3) and confirmation of its suitability for further study of the effect of VaaMPIII-3 on blood and its potential use in the development of new antithrombotic drugs.

Recombinant VaaMPIII-3

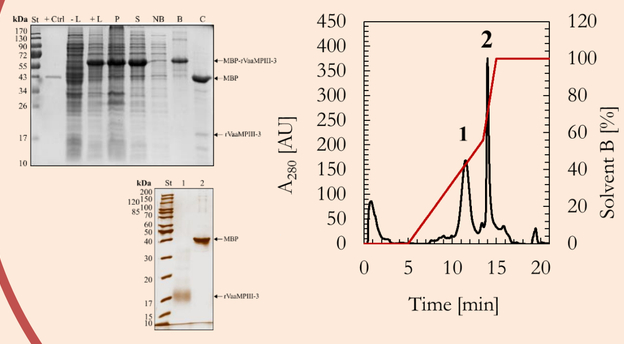
Design



Expression system: *E. coli* ER2523
Vector: pMal-c5X

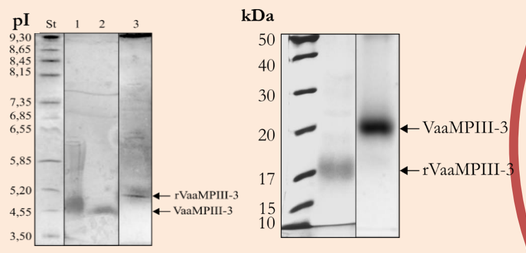
We expressed Maltose Binding Protein (MBP)-tagged rVaaMPIII-3 in *E. coli* and isolated this fusion protein from bacterial lysate by amylose affinity chromatography. After proteolytic removal of the MBP-tag, we purified rVaaMPIII-3 by RP HPLC. To check if the recombinant protein is equivalent to its natural form, we compared their biochemical, biophysical structural, and functional properties.

Expression, isolation, cleavage and purification



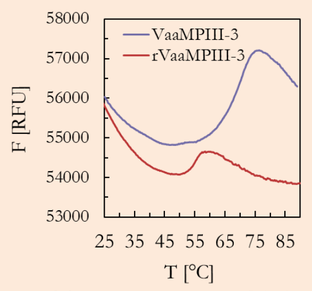
The purity of the isolated 17 kDa rVaaMPIII-3 was confirmed by SDS-PAGE analysis and mass spectrometry.

Biochemical comparison



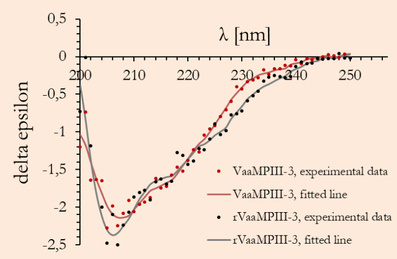
	VaaMPIII-3	rVaaMPIII-3
Molecular mass	21 kDa	17 kDa
Isoelectric point	4.5-5.1	5.1

Biophysical comparison



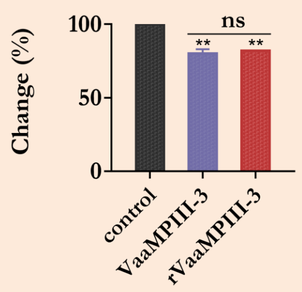
Melting temperature (Tm) of rVaaMPIII-3 (55,57 °C) is lower than Tm VaaMPIII-3 (70,15 °C).

Structural Comparison



Comparison of the CD spectra of natural and recombinant VaaMPIII-3 showing their structural similarity.

Functional comparison



Recombinant and natural VaaMPIII-3 have the same efficacy in inhibiting ADP-induced platelet aggregation.

CONCLUSIONS

- We produced a correctly folded and functional recombinant VaaMPIII-3 for use in further detailed studies of its effect on platelet aggregation and discovering of additional biological activities. as not to depend on the scarcely available snake venom.
- Natural and recombinant VaaMPIII-3 differ in molecular mass, pI, and stability, due to absence of N-glycosylation in the latter.
- Recombinant protein has a similar secondary structure to the natural protein and inhibits APD-induced platelet aggrgation with the same efficacy as its natural form.
- Our further research using rVaaMPIII-3 will help better understand the role of VaaMPIII-3 in snake venom and the underlying mechanism of its actions.

REFERENCE

[1]Leonardi A, Sajevic T, Pungerčar J, Križaj I. Comprehensive Study of the Proteome and Transcriptome of the Venom of the Most Venomous European Viper: Discovery of a New Subclass of Ancestral Snake Venom Metalloproteinase Precursor-Derived Proteins. J Proteome Res. 2019;18(5):2287-2309.

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