Toxicon 79 (2014) 19–27

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Molecular cloning, expression and characterization of albolamin: A type P-IIa snake venom metalloproteinase from green pit viper (*Cryptelytrops albolabris*)

Panchalee Jangprasert, Ponlapat Rojnuckarin*

Department of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Bangkok 10330, Thailand

ARTICLE INFO

Article history:
Received 11 July 2013
Received in revised form 16 December 2013
Accepted 19 December 2013
Available online 28 December 2013

Keywords:
Cryptelytrops albolabris
Snake venom metalloproteinase
Disintegrin
Platelet aggregation
Collagen degradation
Albolamin

ABSTRACT

Snake venom metalloproteinases (SVMPs) can damage vessel wall, degrade clotting factors, inhibit integrins and block platelet functions. Studying them not only gives us deeper insights in pathogenesis of snakebites, but also potentially yields novel therapeutic agents. Here, we discovered a clone of an RGD-containing SVMP from the green pit viper (*Cryptelytrops albolabris*) venom gland cDNA library. Sequence analysis revealed that it belonged to the P-IIa subclass of SVMP comprising signal peptide, prodomain, metalloproteinase and disintegrin. Compared with other P-II SVMPs, it contained 2 additional conserved cysteines that were predicted to prevent the release of disintegrin from the metalloproteinase domain in the mature protein. The N-terminal histidine-tagged construct of metalloproteinase and disintegrin domains of albolamin was inserted into the pPICZαA vector and expressed in *Pichia pastoris*. The recombinant protein molecular weight was approximately 35 kDa on Western blot probed with anti-polyhistidine antibody. The recombinant albolamin could digest human type IV collagen starting within 15 min after incubation. In addition, it dose-dependently inhibited collagen-induced platelet aggregation with the IC50 of 1.8 μM. However, there was no effect on ADP-induced platelet aggregation. Therefore, the inhibition mechanism is probably through blocking collagen receptor(s). Albolamin activities probably contributed to pathology of green pit viper bites. Its disintegrin domain deserves further studies for the potential to be a useful agent affecting platelet functions.

1. Introduction

Green pit viper, *Cryptelytrops albolabris* is the main species responsible for snakebites in Bangkok (Mahasandana and Jintakune, 1990). Its venom exerts many effects on hemostatic system resulting in hemorrhagic symptoms in patients (Mitrakul and Impun, 1973). In addition to hypofibrinogenemia and thrombocytopenia, green pit viper can cause local tissue damages resulting in pain, swelling and/or tissue necrosis (Rojnuckarin et al., 1998). Antivenom therapy can promptly neutralize systemic effects of viper venom, but tissue necrosis still occurs after antivenom treatment (Chotenimitkhun and Rojnuckarin, 2008). The main mechanism of tissue necrosis is due to the catalytic activity of snake venom metalloproteinases (SVMPs) that degrade extracellular matrix proteins and stimulate inflammation causing further damages around biting wounds (Laing et al., 2003). In addition, SVMPs may contribute to systemic bleeding by damaging vascular wall basement membrane and inducing endothelial cell apoptosis, as well as exerting fibrinogenolytic and fibrinolytic activities (Fox and Serrano, 2005; Gutiérrez et al., 2005;

* Corresponding author. Division of Hematology, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand. Tel.: +66 22564564; fax: +66 22539466.
E-mail addresses: rojnuckarinp@gmail.com, porpiasod@hotmail.com (P. Rojnuckarin).

0041-0101/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxicon.2013.12.005

```
tcacgtatgggttgaaagcaggaagagattgcctgtcttccagccaaatccagcctccaaa
atgatccaagttcttttggtaaccatatgcttagcagtttttccttatcaagggagttct
 M  I  Q  V  L  L  V  T  I  C  L  A  V  F  P  Y  Q  G  S  S
ataatcctggaatctgggaacgttaatgattatgaagtcgtgtatccacgaaaagtcact
 I  I  L  E  S  G  N  V  N  D  Y  E  V  V  Y  P  R  K  V  T
ttattacccaaaggagcaattcagccaaagtatgaagacaccatgcaatatgaatttaag
 L  L  P  K  G  A  I  Q  P  K  Y  E  D  T  M  Q  Y  E  F  K
gtgaatggagagccagtgatccttcacctggaaaaaaataaaggacttttttcagaagat
 V  N  G  E  P  V  I  L  H  L  E  K  N  K  G  L  F  S  E  D
tacagcgagactcattattcccctgatggcagagaaattacaacataccccctcggttgag
 Y  S  E  T  H  Y  S  P  D  G  R  E  I  T  T  Y  P  S  V  E
gatcactgctattatcatggacgcatccagaatgatgctgacttaactgcaagcatcagt
 D  H  C  Y  Y  H  G  R  I  Q  N  D  A  D  L  T  A  S  I  S
gcatgcaatggtttgaaaggacatttcaagcttcaaggggagacgtactttattgaaccc
 A  C  N  G  L  K  G  H  F  K  L  Q  G  E  T  Y  F  I  E  P
ttgaagatttccgacagtgaagcccacgcagtctacaaatatgaaaacatagaaaagcag
 L  K  I  S  D  S  E  A  H  A  V  Y  K  Y  E  N  I  E  K  Q
gacgaggcctccaaaatgtgtggggtaaccgagactaattgggaatcagatgaacccatc
 D  E  A  S  K  M  C  G  V  T  E  T  N  W  E  S  D  E  P  I
aaaaagacctcacagttaaatcttactcctgacgaaaaaagattcattgagcttgtcata
 K  K  T  S  Q  L  N  L  T  P  D  E  K  R  F  I  E  L  V  I
attgcggaccacagaatgtacacgaaatatgaaggtgatgagactgagatatgttcaaga
 I  A  D  H  R  M  Y  T  K  Y  E  G  D  E  T  E  I  C  S  R
atatatgaaagtgtcaacagtttaaatttgattttcagagctttgtatattactatagct
 I  Y  E  S  V  N  S  L  N  L  I  F  R  A  L  Y  I  T  I  A
ctgattggcgtagaaatttggtccagcggagatttgatgcctgtgacattatcagcagat
 L  I  G  V  E  I  W  S  S  G  D  L  M  P  V  T  L  S  A  D
gagactttggagtcatttggagaatggagaagaagacattttctgaagagcaaaagacat
 E  T  L  E  S  F  G  E  W  R  R  R  H  F  L  K  S  K  R  H
gataatgctcagttactcacgggcatgatcttcaatgaaaaaatcgaaggaagggcttac
 D  N  A  Q  L  L  T  G  M  I  F  N  E  K  I  E  G  R  A  Y
agaaagagcatgtgtgacccgaagcgttctgtaggaattgttcaagatcatagaagtaga
 R  K  S  M  C  D  P  K  R  S  V  G  I  V  Q  D  H  R  S  R
tatcattttgttgcaaatagaatggcccatgagctgggtcataatctgggcattcatcat
 Y  H  F  V  A  N  R  M  A  H  E  L  G  H  N  L  G  I  H  H
gacggagattcctgtacttgcggtgctaacacatgcattatgtctgaaacagtaagcaac
 D  G  D  S  C  T  C  G  A  N  T  C  I  M  S  E  T  V  S  N
gaaccttccagtcggttcagcgattgtagtcttaatcaatatttgagtgatattattcat
 E  P  S  S  R  F  S  D  C  S  L  N  Q  Y  L  S  D  I  I  H
attcatcgttgccttttgaatgaaccctcgaagacagatattgtttcacctccagtttgt
 I  H  R  C  L  L  N  E  P  S  K  T  D  I  V  S  P  P  V  C
ggcaattactatgtggaggtgggagaagattgtgactgtggccctcctgcaaattgtcag
 G  N  Y  Y  V  E  V  G  E  D  C  D  C  G  P  P  A  N  C  Q
aatccatgctgtgatgctacaacctgtaaactgacaccagggtcacaatgtgcagaagga
 N  P  C  C  D  A  T  T  C  K  L  T  P  G  S  Q  C  A  E  G
ctgtgttgtgcccagtgcaaatttatagaagcaagaaaaatatgtcggaaaggaaggggt
 L  C  C  A  Q  C  K  F  I  E  A  R  K  I  C  R  K  G  R  G
gataatccggatgatcgttgcactggccaatctggtgactgtcccagaaattcctaaaca
 D  N  P  D  D  R  C  T  G  Q  S  G  D  C  P  R  N  S  -
acaatggagacagaatggtctgcaacagcaacaggcagtgttggatggactacagcctac
tacttatcaacctctggcttctctcagattgatttggagaccctgttccagaagattcaa
cttccctccaggtccaaagagaaccatcctgcctgcatcttactagtaaatcactcttag
cttccagaaggcatccaaaaatttattctcccatatttaatctgttttacctcttgctgt
aatcaaacctttcctgccacaaagcttatttgctgtcaaaaaaaaatggcccatttacc
gtttgccaattgccaagcacatttaatgccaacaaattctgggcttttgagctggtgtat
tcgggagtccaatgctacctttccggaaattcatgctggctttcccaaaagtagctgtt
tcccatccaataaactaattattttcattctgaaaaaaaaaaaaaaaaa
```