Toxicon 125 (2017) 1–12

Contents lists available at ScienceDirect

Toxicon

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

# Biochemical and kinetic evaluation of the enzymatic toxins from two stinging scyphozoans *Nemopilema nomurai* and *Cyanea nozakii*

Yang Yue [a, b], Huahua Yu [a, **], Rongfeng Li [a], Ronge Xing [a], Song Liu [a], Kecheng Li [a], Xueqin Wang [a], Xiaolin Chen [a], Pengcheng Li [a, *]

[a] Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, China
[b] University of the Chinese Academy of Sciences, 19 Yuquan Road, Beijing 100039, China

ARTICLE INFO

*Article history:*
Received 17 September 2016
Received in revised form
17 October 2016
Accepted 3 November 2016
Available online 4 November 2016

*Keywords:*
Jellyfish venom
Enzymatic toxins
Enzyme kinetics
Metalloproteinase
*Nemopilema nomurai*
*Cyanea nozakii*

ABSTRACT

Jellyfish envenomations are emerging as an important public health concern occurred worldwide. In China, the situation is getting worse with numerous people stung by jellyfish *Nemopilema nomurai* (*N. nomurai*) and *Cyanea nozakii* (*C. nozakii*) in the summer. However, the proteinaceous mixtures in nematocysts responsible for the symptoms of jellyfish stings were scarcely characterized and understood in view of enzymatic constituents and toxicity. In the present study, enzymatic properties of jellyfish *N. nomurai* and *C. nozakii* nematocyst venom were analyzed biochemically and kinetically. The current data revealed that *N. nomurai* and *C. nozakii* nematocyst venom exhibited various enzymatic activities, of which metalloproteinases activity and $PLA_2$s-like activity were predominant. Moreover, the catalytic activities of metalloproteinases and $PLA_2$s-like were dependent on different physiochemical conditions such as temperature, pH and divalent ions. Kinetic profiling revealed their catalytic behaviors fitted the Michaelis-Menten equation under specific conditions. Findings suggested jellyfish nematocyst venom possessed diverse enzymatic constituents, which may underlie the extensively characterized bioactivities of jellyfish venom and human envenomations. Hence, our study will contribute to understanding the enzymatic constituents and toxicity of jellyfish nematocyst venom and may afford potential therapeutic targets for developing drugs for jellyfish stings.



## 1. Introduction

Scyphozoans, a member of the phylum Cnidaria, are extensively distributed in all oceans and seas of the world from shallow coastal waters to deep seas (Kaartvedt et al., 2015; Kawabata et al., 2013; Sweetman et al., 2014). Most jellyfish inhabit in shallow coastal waters, and therefore many local human beings including fishermen, swimmers and tourists are exposed to jellyfish. Jellyfish stings are troublesome problems usually leading to local cutaneous manifestations characterized by acute pain and inflammatory reactions (Kawahara et al., 2006; Pyo et al., 2016; Silfen et al., 2003). Although scyphozoans are considered to be less potent than cubozoans, in few cases the victims stung by a kind of scyphozoan such as *Nemopilema nomurai* may result in severe systemic symptoms and even suffer from death. Jellyfish venom extracted from nematocysts, mixtures of proteins and peptides, underlie the symptoms induced by jellyfish stings. Jellyfish nematocyst venom was highly complex and varied with diet and geographical distribution (Underwood and Seymour, 2007; Winter et al., 2010). And the biochemical properties of these venom cocktails were poorly understood, which hampered our insights into their toxicological effects.

Enzymatic toxins produced or secreted by various venomous animals from terrestrial or marine source largely consist of metalloproteinases, phospholipases $A_2$ ($PLA_2$s), serine proteases and L-amino acid oxidases (LAAOs) and so on. In venomous animal kingdom, snakes are the most studied creatures and enzymatic toxins from their venom arsenals account for ~ 23.9% of snake venom proteins according to the statistics in animal toxin annotation project (http://www.uniprot.org/program/Toxins). Correlation between enzymatic toxins such as metalloproteinases and $PLA_2$s and biological activities *in vivo* and *in vitro* is observed in some snake venom studies (de Souza et al., 2015; Mackessy, 2010; Zychar et al., 2010) and in general enzymatic toxins is considered to be

* Corresponding author.
** Corresponding author.
*E-mail addresses:* yuhuahua@qdio.ac.cn (H. Yu), pcli@qdio.ac.cn (P. Li).

http://dx.doi.org/10.1016/j.toxicon.2016.11.005
0041-0101/

**Abbreviations**

| | |
|---|---|
| NnNV | jellyfish *Nemopilema nomurai* nematocyst venom |
| CnNV | jellyfish *Cyanea nozakii* nematocyst venom |
| $PLA_2$s | phospholipases A2 |
| LAAOs | L-amino acid oxidases |
| JnVMPs | jellyfish nematocyst venom metalloproteinases |
| NOBA | 4-nitro-3-octanoyloxybenzoic acid |
| 5′-AMP | 5′-monophosphate disodium salt |
| dl-BAPNA | $N_\alpha$-Benzoyl-$D_L$-Arginine-p-Nitroanilide |
| BMT | batimastat |
| UA | ursolic acid |
| *p*-BPB | *p*-bromophenacyl bromide |
| EDTA | ethylenediaminetetraacetic acid |
| EGTA | ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid |
| 1,10-PHE | 1,10-phenanthroline |
| PMSF | phenylmethanesulfonyl fluoride |
| DTT | dithiothreitol |
| Et | ethanol |
| PLDs | phospholipases D |
| SDS | sodium lauryl sulfate |
| PAGE | polyacrylamide gel electrophoresis |
| Tris | tris(hydroxymethyl)aminomethane |
| PBS | phosphate buffered saline |

responsible for immobilization and digestion of prey (Kang et al., 2011). Likewise, the main proteinaceous mixtures of jellyfish nematocyst venom could be divided into enzymatic and non-enzymatic toxins as indicated by extensively experimental or proteomic data (Chung et al., 2001; Helmholz et al., 2007; Lee et al., 2011; Li et al., 2014a; Liu et al., 2015; Ponce et al., 2016; Rachamim et al., 2015; Weston et al., 2013). No-enzymatic jellyfish toxins such as CfTX-1/2 (Brinkman and Burnell, 2007, 2008), CaTX-A/B (Nagai et al., 2000b), CqTX-A (Nagai et al., 2002) and SmP90 (Li et al., 2012) have been isolated and investigated for hemolytic activity, cytotoxic activity and antioxidant activity. The enzymatic components in jellyfish venom were, however, relatively scarcely investigated. In very earlier research, attempts were made to reveal the enzymatic activity of *Stomolophus meleagris* venom using different substrates (Toom and Chan, 1972). Afterwards, $PLA_2$s-like activity was exclusively detected and compared between different cnidarians using the venom extracted from tentacle homogenates rather than isolated nematocysts (Grotendorst and Hessinger, 2000; Nevalainen et al., 2004; Talvinen and Nevalainen, 2002). Relatively high $PLA_2$s activities were detected in either tentacle homogenates or nematocyst extracts of various Scyphozoa species including *Cyanea capillata*, *Cyanea lamarckii* (Pe'ron & Le'slieur) (Helmholz et al., 2007). An interesting research localized a putatively new $PLA_2$ exclusively distributed on outer surface of the undischarged nematocyst of *Rhopilema nomadica* (Lotan et al., 1996). All of the preliminary reports suggest that various enzymatic toxins may exist in jellyfish nematocyst, but their biochemical properties and functional role in biological activities observed and sting-induced envenomations are still elusive.

Recent study involving jellyfish metalloproteinases indicated a positive correlation between cytotoxicity and proteolytic activity (Lee et al., 2011). Another research in regard to delayed jellyfish envenomation syndrome (DJES) revealed the effectiveness of a metalloproteinase inhibitor, BB-94, to treat hemorrhagic injuries induced by jellyfish tentacle extracts (TE) in rat model (Wang et al., 2015). These two studies highlight the necessity to understand the jellyfish nematocyst venom metalloproteinases (JnVMPs). Snake venom metalloproteinases (SVMPs) had been considered to be a new source of valuable enzymes with medical relevance, metalloproteinases from jellyfish, however, were not yet characterized and understood for their biochemical properties. In the course of targeting the active compounds behind jellyfish sting-induced envenomations, comprehensive analysis into proteomic and transcriptomic data of two scyphozoans, *N. nomurai* and *Cyanea nozakii Kishinouye,* revealed the possible existence of various enzymes including metalloproteinases and $PLA_2$s (Li et al., 2014a, 2016). And these toxins were assumed to be associated with jellyfish envenomations. The present study is designed to experimentally analyze and compare the enzymatic properties of *N. nomurai* and *C. nozakii* nematocyst venom including optimal catalytic conditions, stability and effects of class-specific inhibitors, and emphasis is given on metalloproteinases and $PLA_2$s. In addition, enzyme kinetics of JnVMPs and $PLA_2$s are firstly explored with their respectively chromogenic substrates. The introduction of enzyme kinetics into jellyfish venom will offer an alternative way to reveal the chemical nature and toxicity of jellyfish venom. Moreover, deciphering the enzymatic constituents will facilitate understanding the molecular mechanism of jellyfish stings and provide important references for developing drugs for human envenomations.

## 2. Material and methods

### 2.1. Chemical and regents

Azocasein, batimastat (BMT), ursolic acid (UA), $N_\alpha$-Benzoyl-$D_L$-Arginine-*p*-Nitroanilide (DL-BAPNA), *p*-bromophenacyl bromide (*p*-BPB), L-leucine, o-phenylenediamine, peroxidase, adenosine 5′-monophosphate disodium salt (5'-AMP) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 4-nitro-3-octanoyloxybenzoic acid (NOBA) was from Abcam (Shanghai, China). Triton X-100 and trypsin 1:250 (tissue culture grade, trypsin >250 N.F.U/mg) were purchased from Solarbio Inc. (Beijing, China). All other regents used were of analytical grade.

### 2.2. Sample collection

The jellyfish specimens *N. nomurai* and *C. nozakii* were collected from Aoshan Bay in Qingdao, China in August 2013, 2016. The fishing tentacles of both scyphozoans were excised manually from live specimens and pooled together into plastic zip-pack bags for storage at − 80 °C. In China, jellyfish blooming occurs almost every summer and has a growing trend. Since jellyfish is not an endangered or protected species in China, so fishing for jellyfish is permitted by the department of fisheries.

### 2.3. Isolation of nematocysts and venom extraction

For two jellyfish specimens, nematocysts were isolated from the fishing tentacles as described in the literature (Bloom et al., 1998; Carrette and Seymour, 2004) with slight modifications. Briefly, the frozen tentacles were thawed in seawater at 4 °C for autolysis. Water exchanges were performed every 24 h for 4 days. After autolysis, the mixtures were filtered through a 200-mesh plankton net to remove the debris. The filtrate was then centrifuged at 940 g