Biochemical and Biophysical Research Communications 387 (2009) 617–622

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Consensus sequence L/PKSSLL mimics crucial epitope on Loop III of Taiwan cobra cardiotoxin

Ping-Chieh Wang [a], Kah-Sin Loh [a], Shih-Ting Lin [a], Tzu-Ling Chien [a], Jen-Ron Chiang [b], Wen-Chin Hsieh [b], Bor-Lin Miao [b], Cheng-Fu Su [b], Wen-Jen Yang [a,*]

[a] Institute of Biotechnology, National University of Kaohsiung, Kaohsiung 811, Taiwan
[b] Vaccine Center, Centers for Disease Control, Taipei 115, Taiwan

ARTICLE INFO

Article history:
Received 13 July 2009
Available online 24 July 2009

Keywords:
Phage display
Epitope
Cardiotoxin
Taiwan cobra (*Naja naja atra*)

ABSTRACT

Phage display is effective in screening peptides that mimic venom's neutralizing epitopes. A phage display cyclized heptapeptide library (C7C library) was panned with purified divalent antivenin IgG, which neutralizes *Naja naja atra* venom (NAV) and *Bungarus multicinctus* venom (BMV). The selected heptapeptide sequences were aligned with known protein sequences of NAV and BMV in GenBank. One of the four consensus sequences, L/PKSSLL, mimicked the crucial epitope on Loop III of Taiwan cobra cardiotoxin that is associated with the venom's lethal potency. In dot blot analysis, several clones showed varying reactivities for NAV monovalent antivenin and lesser cross-reactions with BMV monovalent antivenin. The KSSLLRN-carrying phage occurred four times in selected clones and showed the strongest reactivity to NAV monovalent antivenin. Furthermore, the QDSLLPS-carrying phage also presented significant dot blot signal, indicating that the SLL sequence shared by these two clones may be a crucial antibody-binding site.

© 2009 Elsevier Inc. All rights reserved.

Introduction

Snakebite is a serious global public health issue, especially in numerous tropical and subtropical countries like Taiwan. Taiwan cobra (*Naja naja atra*) is a crucial venomous snake and causes about 10% of all snakebite incidents in Taiwan [1]. Snake venoms are a complex mixture of many diverse toxins. Cobrotoxin, cardiotoxins, and phospholipase A_2 (PLA_2) are the three major toxic proteins of Taiwan cobra venom [2]. Among these, cobrotoxin is the most predominant neurotoxin. It is a small, basic protein consisting of a single polypeptide chain with 62 amino acids, cross-linked by four disulfide bonds [3]. Cobrotoxin binds to the nicotinic acetylcholine receptor on postsynaptic membranes with high binding affinity and blocks neuromuscular transmission, leading to muscle contraction dysfunction [4]. Cardiotoxins are composed of 60–63 amino acids (molecular weight 6.5–7.0 kDa) in a single β-sheet polypeptide chain, cross-linked by four disulfide bonds [5]. Interestingly, although cobrotoxin and cardiotoxin are similar in their 3D structures, they present very different biological activities. Cardiotoxin can produce depolarization of nerve and muscle cells to affect the contraction of the cardiac muscle, induce cancer cell apoptosis, and cause lysis of erythrocytes and epithelial cells [2,5,6]. To date, several cardiotoxin isoforms have been isolated and characterized from the venom of the Taiwan cobra (*N. naja atra*) [5,7]. Comparison of the lethal potency and 3D structure(s) of these cardiotoxin isoforms from Taiwan cobra venom has shown that the lethal potency of cardiotoxins strongly correlates with the presence of a nonpolar "finger-shaped" projection that comprises of hydrophobic residues Leu47 and Leu48 at the tip of Loop III. It was predicted that this finger-shaped projection forms a part of the putative receptor binding site of cardiotoxins [8]. Snake PLA_2s are a group of polypeptides, about 120–130 amino acids in length, and each chain is cross-linked by seven disulfide bonds [9]. Venom PLA_2 has been shown to possess various pharmacological effects such as cardiotoxic, myonecrotic, neurotoxic, hemolytic, and anticoagulant actions in addition to its enzymatic activity in the hydrolysis of ester bonds in phosphoglycerides [10]. It has been shown that both His-47 and Asp-93 are essential for the catalytic activity of PLA_2 from Taiwan cobra [11].

To date, antivenin administration is the world's major therapy for snakebites. In Taiwan, a pepsin-digested $F(ab')_2$ bivalent antivenin produced from equine serum by the National Institute of Preventive Medicine has been used to treat cobra snakebites since 1986, and it has shown a very low risk of acute adverse reactions [12]. A local antivenin injection can speed up neutralization and reduce the spread of venom. The efficacy of antivenin is strongly correlated with the neutralizing antibodies against the epitopes

* Corresponding author. Address: Institute of Biotechnology, National University of Kaohsiung, 700, Kaohsiung University Road, Nanzih District, Kaohsiung 811, Taiwan. Fax: +886 7 5919404.
E-mail address: wjyang@nuk.edu.tw (W.-J. Yang).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.07.097

of venom that are crucial for its lethal potency. The phage display random peptide library has emerged as a powerful tool for analyzing antigen–antibody interactions and is successfully used to mimic epitopes of antigens (mimotopes) [13]. Peptides mimicking two epitopes of neuwiedase from *Bothrops neuwiedi* snake venom were found; these mimotopes could induce antibodies that efficiently recognized the toxin [14].

In this study, a phage display cyclized heptapeptide library (C7C library) was used to identify peptides that bind to protein A-purified antivenin IgGs that can neutralize Taiwan cobra venom. A major clone that displayed KSSLLRN and a consensus sequence, L/PKSSLL, which mimicked the epitope on the Loop III of cardiotoxin of Taiwan cobra that is associated with its lethal potency, were identified These data of epitopes can provide valuable information for snake venom studies in diagnosis and treatment.

Materials and methods

Materials. A phage display cyclized heptapeptide library (C7C library) was purchased from New English Biolabs, USA. The library has a pair of cysteine residues flanking the random heptapeptide, resulting in phage display of cyclized peptides, was fused to the minor coat protein III at the N-terminus of M13 phage. Taiwan cobra (*N. naja atra*) venom (NAV) and *Bungarus multicinctus* venom (BMV) were kindly provided from the Vaccine Center, Centers for Disease Control (CDC), Taiwan. The HRP/anti-M13 monoclonal antibody conjugate was purchased from GE Healthcare Inc.

Animals and antivenin preparation. An equine-derived monovalent antivenin against NAV and a bivalent antivenin against NAV and BMV were kindly provided by the Vaccine Center, CDC, Taiwan. The BMV antivenin used in this study was prepared by subcutaneous injection of glutaraldehyde (GA)-detoxified venom mixed with Freund's adjuvant in mice. Inbred specific pathogen-free (SPF) BALB/c female mice were purchased from the National Laboratory Animal Center (Taipei, Taiwan). All experiments were performed in accordance with institutional guidelines. The venom was detoxified by 0.125% glutaraldehyde, incubated at room temperature for 30 min, and then at 4 °C overnight. Twelve-weeks-old mice were immunized with 20 μg detoxified BMV for each animal. The Freund's complete adjuvant was administered in the prime injection, and the incomplete adjuvant was used in the subsequent boosters at 2-week intervals. The animals were bled 1 week after the third injection. The titers of antibodies were measured by ELISA using serial dilutions of antisera against BMV. The antibody titer was defined as the reciprocal of the maximum dilution factor of the test serum that kept the OD_{405} reading above 0.2.

Purification and characterization of equine antivenin. The equine-derived bivalent antivenin with a potency of 80 antitoxic units against NAV was obtained from the Vaccine Center, CDC, Taiwan. To better perform the biopanning process, IgGs of the antivenin were purified using a protein A purification method, modified from [15]. Briefly, a protein A-agarose resin packed column was washed with 10 column-volumes of starting buffer (100 mM Tris–HCl, pH 7.5, 100 mM NaCl). The antivenin sample was mixed with an equal volume of starting buffer before being applied to the column. The pass-through solution was collected while measuring OD_{280}. The column was washed with starting buffer until OD_{280} reduced to background levels. The IgG was eluted with 0.1 M glycine–HCl (pH 2.5), immediately neutralized with 1 M Tris–HCl (pH 8.0), and stored at −20 °C until use. The concentration of eluted IgG was estimated by OD_{280} (1 OD_{280} = 0.75 mg/ml). Western blot analysis and potency test were used to further characterize the eluted IgG against NAV [16]. The potency test was performed according to the standard operation procedure for antivenin antitoxin activity measurement at Vaccine Center, CDC, Taiwan.

Biopanning, DNA sequencing, and sequence analysis. The biopanning of purified IgG with the C7C library was performed as described previously [15]. Small aliquots of the eluted phage were used for determining the phage titer. The remaining elute was used to infect *Escherichia coli* ER2738 for phage amplification. After three rounds of biopanning, the DNA sequences of randomly selected phage clones were determined by a fluorescence-based sequencer. The sequences of phage-displayed heptapeptides were deduced from the DNA sequences and were further grouped according to the amino acids that were identical at the position of alignment. Alignments of these sequences with the known protein sequences of NAV and BMV in GenBank were also performed by the BLAST software to understand whether the sequences mimic the epitopes on these venom proteins The amino acid sequences of eight cardiotoxin isoforms from Taiwan cobra venom, CTX-3 (NCBI Accession Number: U42585), CTX-1 (U42583), CTX-2 (U58485), CTX-4 (Y12491), CTX-5 (U58489), CTX-8 (U42586), CTX-10 (Y18957),

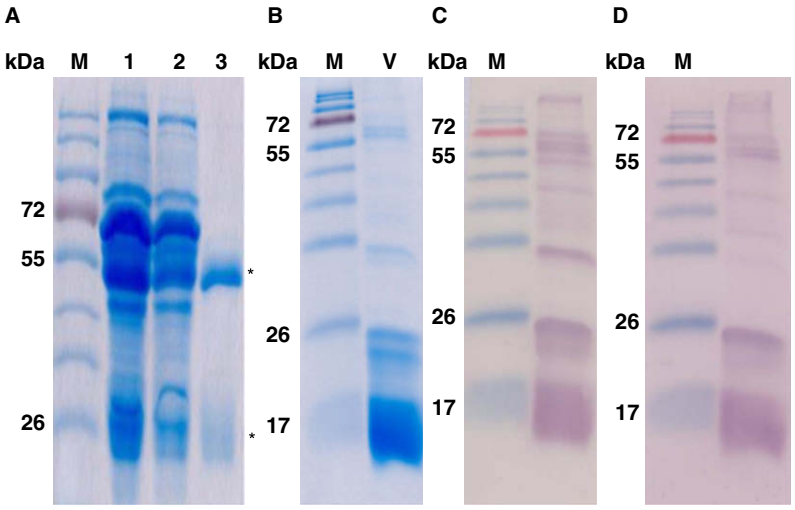

Fig. 1. Characterization of snake venoms and antivenin. (A) SDS–PAGE analysis of bivalent antivenin against NAV and BMV. Lane M: protein marker (the molecular weight is indicated on the left); lane 1: unpurified divalent antivenin; lane 2: the collection of unbound fractions of divalent antivenin from protein A affinity column; lane 3: protein A-purified IgG, the heavy chain (50 kDa) and light chain (25 kDa) of IgG are indicated with an asterisk. (B) SDS–PAGE analysis of NAV. Lane M: protein marker; lane V: 12 μg NAV protein. Western blot analysis using (C) unpurified divalent antivenin or (D) protein A-purified IgG against 12 μg NAV protein.

Download English Version:

https://daneshyari.com/en/article/1933143

Download Persian Version:

https://daneshyari.com/article/1933143

Daneshyari.com