Discovery of Selective Nanobodies against -elapitoxin Dpp2c from Black Mamba through Phage Display Screening

Milbo, Christina; Laustsen, Andreas Hougaard; Lohse, Brian

Publication date:
2015

Document Version
Publisher's PDF, also known as Version of record

FACULTY OF HEALTH AND MEDICAL SCIENCES
UNIVERSITY OF COPENHAGEN

Discovery of Selective Nanobodies against α-elapitoxin Dpp2c from Black Mamba through Phage Display Screening

Christina Milbo[1], Andreas Hougaard Laustsen[2], and Brian Lohse[2]
[1]Department of Systems Biology, Technical University of Denmark, [2]Department of Drug Design and Pharmacology, University of Copenhagen

Targeting black mamba α-neurotoxins with nanobodies

Feared for its highly neurotoxic venom and rapid attack technique, the Black mamba (*Dendroaspis polylepis*) is Africa's largest venomous snake [1]. The clinical manifestations of a bite from *D. polylepis* include flaccid paralysis leading to respiratory failure and death due to post-synaptic blockade of the neuromuscular junctions caused by α-neurotoxins [1-4]. Since antivenoms suffer from a reactivity bias towards larger toxins due the fact that antivenoms are produced by immunization of large mammals, current antivenoms could be reinforced by addition of monoclonal antitoxins directed towards the smaller α-neurotoxins [1,5]. Here, we report the discovery of selective nanobodies targeting α-elapitoxin Dpp2c from *D. polylepis* through phage display screening [6].

Figure 1: *Dendroaspis polylepis* (Black mamba) eating prey. Photo: Ted Arensmeier 2007

Results – two selective nanobody binders discovered

A M13 phage library displaying nanobody genes from a llama immunized with venom from the monocled cobra (*Naja* kaouthia) [7,8] was selected against a venom fraction from *D. polylepis* venom containing high amounts of α-elapitoxin Dpp2c. Two monoclonal phages that bound strongly to this fraction were isolated. Monoclonal phage DNA will soon be sequenced, which will unveil the primary structure of the nanobodies displayed on the phages.

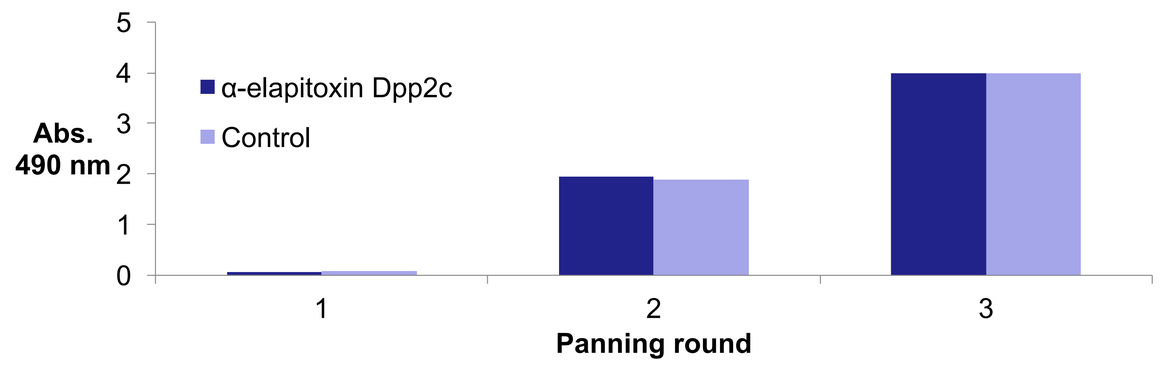


Figure 2: ELISA results. The polyclonal phage library from the third round of panning yields a strong ELISA signal, indicating the presence of strong peptide binders to α-elapitoxin Dpp2c

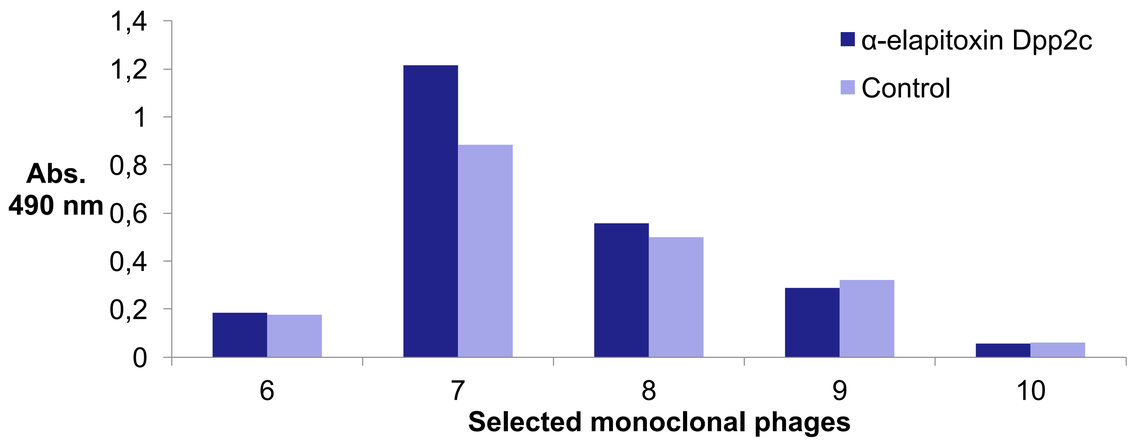
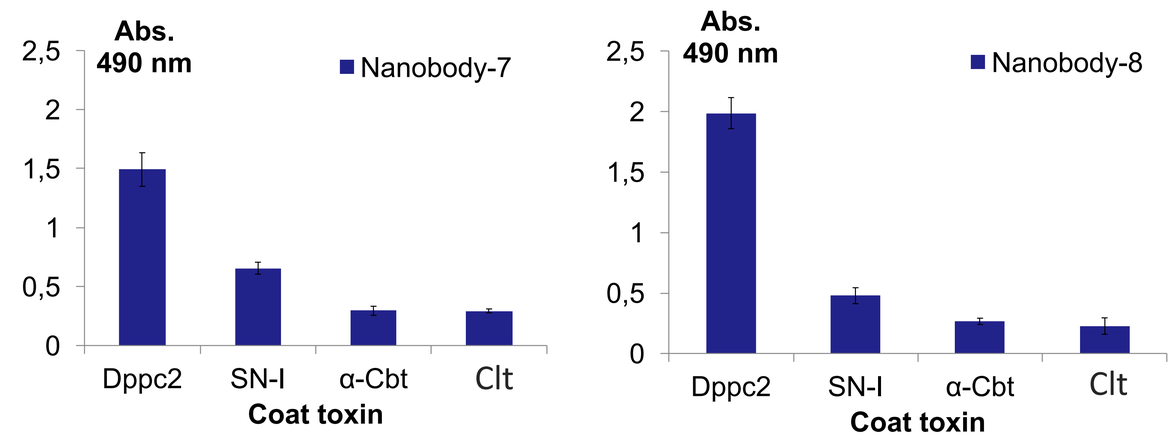


Figure 3: ELISA results for 10 selected phage monoclones against α-elapitoxin Dpp2c.



Figur 4: ELISA-based cross-reactivity study of the nanobody-displaying isolated phages. Dppc2: α-elapitoxin Dpp2c, SN-I: Short neurotoxin I, α-Cbt: α-cobratoxin.

Outlook – Reinforcing antivenoms with nanobodies

The isolated monoclonal nanobody displaying phages showed great selectivity towards α-elapitoxin Dpp2c and could potentially be added to existing antivenoms to reinforce their response towards this lethal α-neurotoxin. Once the sequences of the displayed nanobodies is known, the next steps include biosynthesis, determination of binding constants for the nanobodies, and measurement of their ability to inhibit α-elapitoxin Dpp2c *in vitro* and *in vivo.*

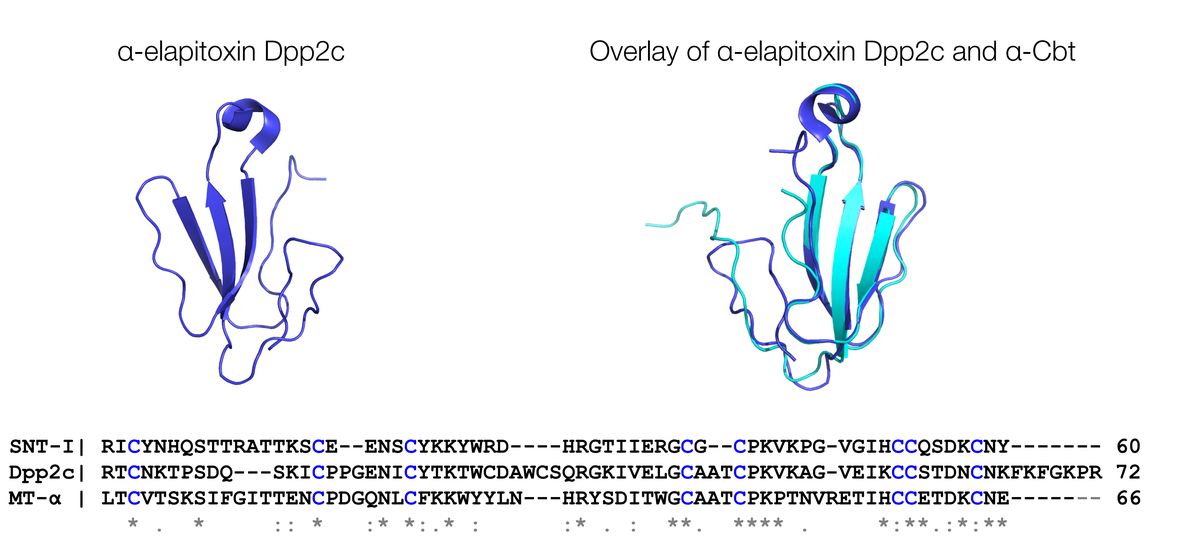


Figure 5: Cartoon model of α-elapitoxin Dpp2c compared with overlaid cartoon models of α-elapitoxin Dpp2c and α-cobratoxin from *Naja kaouthia* (α-Cbt, cyan), drawn in PyMOL. Sequence alignment short neurotoxin I (SNT-I), α-elapitoxin Dpp2c (Dpp2c), and muscarinic toxin-α (MT-α), which are all α-neurotoxins from *D. polylepis*.

References

[1] Laustsen, A. H. et al. (2015). Unveiling the nature of black mamba (Dendroaspis polylepis) venom through venomics and antivenom immunoprofiling: Identification of key toxin targets for antivenom development. *Journal of proteomics*, 119, 126-142.
[2] Wang, C. I. A. et al. (2014). Isolation and structural and pharmacological characterization of alpha-Elapitoxin-Dpp2d, an amidated three finger toxin from black mamba venom. *Biochemistry*, 53, 3758–66.
[3] Blanchet, G. et al. (2014). Polypharmacology profiles and phylogenetic analysis of three-finger toxins from mamba venom: case of aminergic toxins. *Biochimie*,103C, 109–17.
[4] Jolkkonen, M. et al. (2001). Kinetic evidence for different mechanisms of interaction of black mamba toxins MT alpha and MT beta with muscarinic receptors. *Toxicon*, 39, 377–82.
[5] Fernández, J. et al.(2011). Venomic and antivenomic analyses of the Central American coral snake, Micrurus nigrocinctus (Elapidae). *Journal of Proteome Research*, 10, 1816–27.
[6] Petras, D. et al. (2011), Snake venomics of African spitting cobras: toxin composition and assessment of congeneric cross-reactivity of the Pan-African EchiTAb-Plus-ICP® antivenom by antivenomics and neutralization approaches. *Journal of Proteome Research,* 10, 1266–80.
[7] Hoogenboom, H. R. et al. (1998). Antibody phage display technology and its applications. *Immunotechnology*. 4(1), 1-20.
[8] Richard, G. (2013). In vivo neutralization of alpha-cobratoxin with high-affinity llama single-domain antibodies (VHHs) and a VHH-Fc antibody. *PLoS One,* 8, e69495.

Contact information
cmilbo@bio.dtu.dk / (+45) 2216 2807

Acknowledgement
Collaborators: Mikael Engmark, Federico De Masi

Financial support: Department of Drug Design and Pharmacology, University of Copenhagen