IOCT 2025 Conference

The 3rd International Online Conference on Toxins

10–12 September 2025 | Online

MDPI

Epitopes-targeted discovery of aptamer targeting cobra venom cytotoxin

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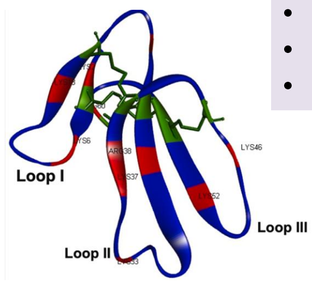
INTRODUCTION & AIM

Cytotoxin (CTX)

- Three-fingered toxin
- Predominant in cobra venom
- Induce dermonecrosis
- Low in immunogenicity

Cobra (*Naja spp.*)



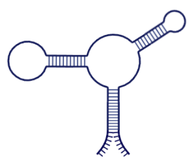
CTX

Ineffective in treating dermonecrosis

Poor neutralisation efficacy and limited cross reactivity

Antivenom

VS



Aptamer

Affordability

Manufacturing feasibility

Safe

Objectives

- Discover aptamers that can specifically recognise these functional epitopes.
- Determination of the binding affinity, neutralisation efficacy and mechanisms of actions of the aptamer candidates.

METHODS

>CTX

LKCNNKLVPLFYKTCPAGKNLCYKMFMVSTPTKVPVKRGCIDVCPKNSLLVKYVCCNTDRCN
Loop I Loop II Loop III

Conserved CTX sequence with 4 functional epitopes: 'KLVPLFYK' (epitope peptide 1; E1), 'AGKNL' (epitope peptide 2; E2), 'MFMVSTPKVPV' (epitope peptide 3; E3) and 'DVCPKNSLL' (epitope peptide 4; E4), were used (Misuan et al., 2023; Hiu et al., 2023).

Computational Analysis

Aptamer Selection

- Apta-Index™ Database
- 15-30 nucleotides
- 30 aptamer candidates

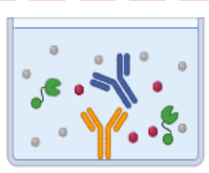
Molecular Docking

- Blind docking between epitope peptides and aptamer candidates
- Site specific docking between epitopes on CTX and aptamers

Computational Analysis

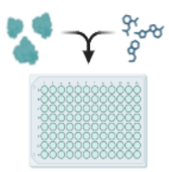
- Docking scores, Z-score and Root Mean Square Median (RMSD) were evaluated
- Intermolecular interactions

Experimental Analysis



Enzyme-Linked Aptamer Assay (ELAA)

- Direct ELAA
- Competitive ELAA



Neutralisation Assay

- In vitro neutralisation
- Experimental post-envenomed model

Mechanism of Action

- RIPK3 assays

RESULTS & DISCUSSIONS

Intramolecular Interactions for Molecular Docking

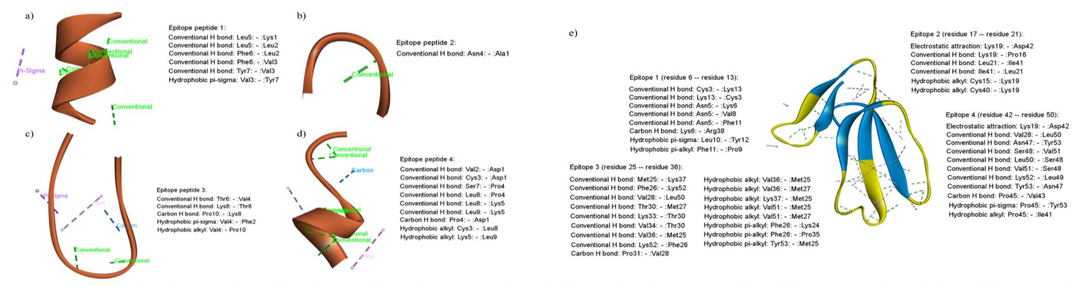


Figure 1: Intramolecular interactions for the predicted structural modelling of epitope peptides (diagram a-d) and CTX (diagram e), created using Biovia Discovery Studio version 2024.

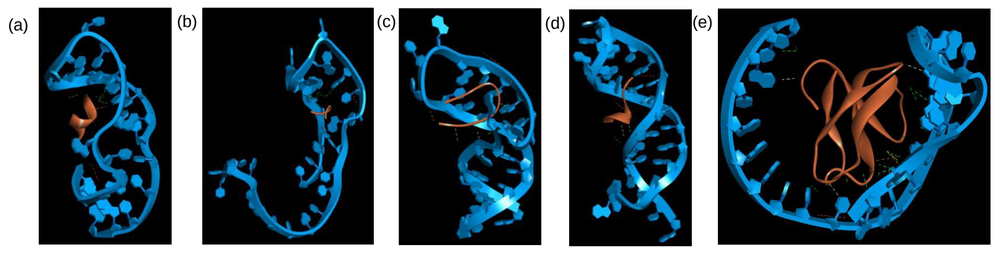


Figure 2: Molecular visualization of the best docking complexes: E1_A15 (a), E2_A33 (b), E3_A26 (c), E4_A31 (d) and CTX_A28 (e), created with Biovia Discovery studio version 2024.

ELAA

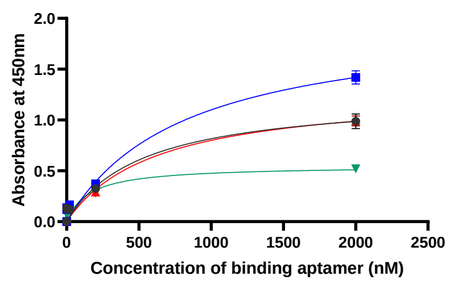


Figure 3: Direct ELAA

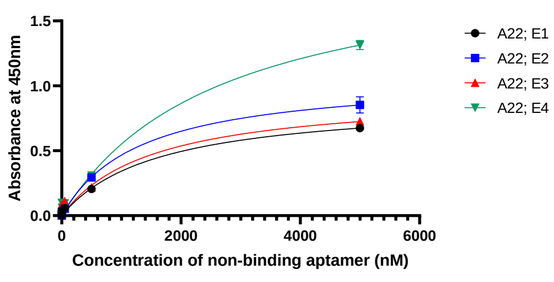


Figure 4: Competitive ELAA

Table 1: Dissociation constant (KD), limit of detection (LOD) and limit of quantification (LOQ) for direct and direct competitive ELAA.

Direct ELAA					Competitive ELAA				
Epitope peptide	Binding Aptamer	K_D (nM)	LOD (nM)	LOQ (nM)	Epitope peptide	Binding Aptamer	K_D (nM)	LOD (nM)	LOQ (nM)
1	15	755.0	22.59	68.22	1	22	1711.0	78.00	236.27
2	33	1166.4	35.90	107.81	2		1440.0	122.13	369.97
3	26	1102.6	15.24	45.91	3		2123.2	151.81	459.85
4	31	187.7	15.29	46.12	4		3278.0	53.27	161.24

Neutralisation & Mechanism of Action

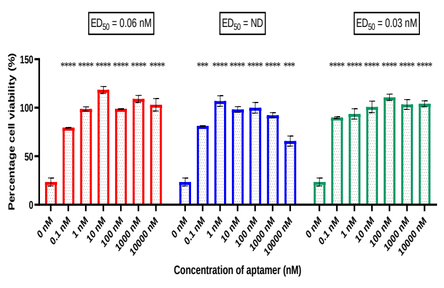


Figure 5: In vitro neutralisation of aptamers and epitope peptides

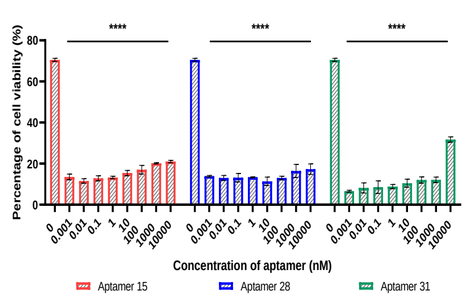


Figure 6: Experimental post-envenomed model neutralisation of aptamers and epitope peptides

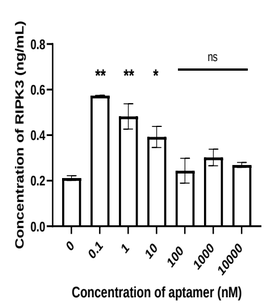


Figure 7: RIPK3 assays

CONCLUSION

- Individual epitope peptides did not cause significant cell death.
- E1 (located in loop I) and E4 (located in loop III) were found to be cytolytic domains that work synergistically in causing cell cytotoxicity.
- Apt 31 could neutralize the synergistic cytotoxicity of E1 and E4 in pre-envenomed condition with an ED_{50} of 0.03nM but not in post-envenomed condition.
- Apt 31 is the most potent candidate in neutralizing cytotoxicity of epitope peptide in pre-envenomed setting.

REFERENCES

Hiu, JJ, Fung, JKY, Tan, HS & Yap, MKK (2023) Unveiling the functional epitopes of cobra venom cytotoxin by immunoinformatics and epitope-omic analyses. *Sci Rep.* 13(1) 12271.

Liu CC, Chou YS, Chen CY, Liu KL, Huang GJ, Yu JS, Wu CJ, Liaw GW, Hsieh CH, Chen CK (2020). Pathogenesis of local necrosis induced by *Naja atra* venom: Assessment of the neutralization ability of Taiwanese freeze-dried neurotoxic antivenom in animal models. *PLoS Neg. Trop. Dis*. 14(2): 1-20.

Misuan N, Mohamad S, Tubiana T, Yap MKK (2023). Ensemble-based molecular docking and spectrofluorometric analysis of interaction between cytotoxin and tumor necrosis factor receptor 1. *J Biomol Struct Dyn*. 41(24):15339-15353.