Deciphering the Biophysical Properties of a Novel Linear Conotoxin: Recombinant Expression and Profiling of Mo1692

D. Zarena
Dept. of Physics, JNTUA College of Engineering, Anantapur - 515002, India
Email: zareenajntua@gmail.com

Abstract: Linear conotoxins from *Conus monile* represent an understudied class of bioactive peptides. Mo1692 is a 15-residue linear peptide (HEGKPGHKSPLDDKF) derived from the natural peptide Mo1705. The biosynthetic peptide contains proline instead of hydroxyproline at position 10, resulting in a 16 Da mass reduction. Mo1692 was successfully expressed in *E. coli* as a cytochrome b5 fusion protein and purified using Ni^{2+}-IDA chromatography and Fast Protein Liquid Chromatography. LC-ESI MS confirmed the purified fusion protein mass of 13975Da. Circular dichroism spectroscopy revealed a well-folded structure. This work establishes a reliable bacterial expression system for linear conotoxins and contributes to understanding the structural diversity of *C. monile* venom peptides. The successful expression and characterization of Mo1692 provides a foundation for further biophysical studies of linear conotoxins from Indian marine cone snails.
Key words: Mo1692, *Conus monile*, Conotoxin, *cytb5* fusion, Chromatography, Mass spectrum, Circular dichroism.
How to cite this article: Zarena D. Deciphering the biophysical properties of a novel linear conotoxin: recombinant expression and profiling of Mo1692. Int J Drug Deliv Technol. 2026;16(72s): 79-84. DOI: 10.25258/ijddt.16.72s.11

Introduction: Conotoxins are small disulfide-rich peptides derived from the venom of marine cone snails belonging to the genus *Conus*. These remarkable bioactive compounds represent one of nature's most sophisticated molecular arsenals, evolved over millions of years for efficient prey capture and predator defense. The venoms of marine cone snails contain a complex mixture of toxin peptides that exhibit extraordinary specificity in targeting ion channels and receptors of excitable cells, making them invaluable tools for neuroscience research and pharmaceutical development [1-3].

The complexity of *Conus* venoms is truly remarkable, with current estimations suggesting that approximately 200 different peptides may be present in a single species' venom and more than 50,000 conotoxins potentially exist across the entire genus *Conus*[4, 5]. This extraordinary diversity represents an untapped reservoir of bioactive molecules with immense therapeutic potential. The high species diversity of cone snails, with approximately 1,000 recognized species worldwide, further amplifies the chemical diversity available for pharmaceutical exploration [6,7].

The structural architecture of conotoxins is characterized by their rigid three-dimensional conformations, which are crucial for their specific interactions with target proteins. These peptides possess well-defined structural frameworks that enable their remarkable selectivity and potency. In the majority of conotoxins, structural stability is maintained through multiple disulfide bonds formed between specific cysteine residues within the peptide sequence. These disulfide bridges create compact, stable structures that are resistant to enzymatic degradation and maintain their bioactivity under physiological conditions. However, while disulfide-rich peptides constitute the predominant class of conotoxins, a considerable number of linear peptides without disulfide bridges are also present in cone snail venoms, representing an underexplored class of bioactive molecules [8].

Despite the wealth of knowledge accumulated regarding disulfide-rich conotoxins, linear peptides from cone snail venoms have received significantly less attention in the scientific literature. These linear peptides represent a distinct class of bioactive molecules that may possess unique structural and functional properties compared to their disulfide-rich counterparts. The absence of constraining disulfide bonds may confer different conformational flexibility, membrane permeability and target selectivity profiles. Understanding the structural diversity and biological activities of linear conotoxins is essential for comprehensive characterization of the complete conotoxin arsenal and may reveal novel therapeutic opportunities [8].

India's extensive coastal waters, particularly along the Tamil Nadu coast, harbor diverse populations of cone snail species, including *Conus monile*, which represent valuable resources for conotoxin discovery. The exploration of conotoxins from Indian marine

cone snails is of particular significance as regional species may have evolved unique peptide variants adapted to local ecological conditions. The systematic investigation of linear peptides from Indian cone snail species may reveal novel structural motifs and biological activities that have not been previously characterized in other geographic populations [9, 10].

The present research work represents a focused investigation into the bacterial expression, purification and comprehensive biophysical characterization of linear peptides derived from Indian marine cone snails. This study aims to illuminate the structural diversity of conotoxins by specifically examining the properties of linear peptides, which have been understudied compared to their disulfide-rich counterparts. Through systematic biochemical and biophysical analyses, this research seeks to expand our understanding of the complete conotoxin repertoire and its therapeutic potential.

To achieve these objectives, we have focused our investigations on a 15-residue linear peptide derived from the marine cone snail *Conus monile* collected from the coastal waters of Tamil Nadu, India. This study focused on bacterial expression, purification and biophysical characterization of this linear conotoxin to better understand its structural properties and contribute to our knowledge of the understudied linear peptide class within the conotoxin family.

Materials & Methods:

Plasmid pET 21, E. coli strains Nova Blue and BL21(DE3) were purchased from Novagen. Oligonucleotides were custom-synthesized and purchased from Sigma–Genosys India. Enzymes for recombinant DNA technology such as Taq polymerase, T4 DNA ligase, NdeI, EcoRI and BamHI were purchased from Bangalore Genei, India. Kits for purification of PCR fragments, plasmid extraction and extraction of restriction fragments separated on an agarose or on a polyacrylamide gel were purchased from Qiagen, Novagen or Sigma–Aldrich. All other chemicals and biochemicals were purchased from Sigma–Aldrich, Calbiochem or Merck India. Ni^{2+}-IDA agarose was purchased from Sigma–Aldrich.

Protein expression: Plasmid containing the gene encoding for the cytochrome b5–Mo1659 fusion protein was used to transform E. coli overexpression strain BL(21)DE3 [11]. Isolated colonies were picked and cultured overnight in LB broth at 37°C. Overnight cultures served as inoculum. Large-scale protein production was accomplished by seeding a 2-L batch (500ml - 4 each in 2L flasks) of growth media with 1% inoculum. Cultures were grown at 37°C with shaking at 200rpm until the cultures reached an OD of 0.6 - 0.8, at which time overexpression of protein was induced by the addition of IPTG to a final concentration of 0.5mM. The cells were then allowed to grow for a further period of 5 h. Cells were harvested by centrifugation (5000rpm, 4°C). The supernatant was discarded and the cells were resuspended in buffer A (TEG - 20mM Tris - HCl, 10mM EDTA and 50mM glucose, pH 8.0) and stored overnight at 80°C. Frozen cells were thawed and lysed using a French press (1100psi). Cell debris was separated from the supernatant by centrifugation (12,000rpm, 4°C, 30min) and discarded. Induction of protein expression was monitored by withdrawing 1 ml samples of culture every hour, post-induction. The cells were pelleted, lysed in Laemmli buffer and analyzed on a 15% SDS–PAGE gel by electrophoresis.

Protein purification: The supernatant from cell lysis was loaded onto a DEAE–Sepharose (Amersham) anion exchange column (40ml bed volume) that had been previously equilibrated with buffer B (20mM Tris-HCl, pH 8.0). The column was then washed extensively overnight (20 column volumes) with buffer B. Protein was eluted from the column by the application of a linear salt (NaCl 0-1M) gradient. Fractions of 5 ml volume were collected and the extent of purification was monitored by SDS–PAGE gel electrophoresis. Fractions that contained the protein were then pooled and further purified using a Ni^{2+}-IDA chromatography step [12], in which the bound protein was eluted from the column by the application of a linear imidazole (0-1M) gradient. Eluted fractions from the Ni^{2+}–IDA column were pooled and dialyzed against 10mM Tris (pH 8.0). Ni^{2+}–IDA column chromatography was followed by gel filtration chromatography (FPLC) for higher level of protein purification.

Mass determination:

Liquid chromatography electrospray ionization (ESI) mass spectra were recorded on a Bruker Daltonics Esquire 3000 Plus Ion Trap Mass Spectrometer attached to an Agilent 1100 series HPLC system. The samples were infused into the mass spectrometer through a reverse-phase C18 column, by application of a gradient elution using a binary solvent delivery system (solvent A - 0.1% formic acid in water and solvent B - 0.1% formic acid in acetonitrile) at a flow rate of 0.3ml/min. The data were acquired over a m/z range of 100–2000 in positive ion mode and analyzed using Esquire analysis software.

Circular Dichroism:

Circular dichroism (CD) spectra were recorded on a Jasco J-715 spectropolarimeter in a quartz cuvette

(1mm path length) at a protein concentration of 10µM in 10mM Tris, 100mM NaCl (pH 7.4) buffer. The CD spectra were acquired over a wavelength range of 190 to 260nm for the far-UV CD spectra. The data were acquired with a scan rate of 100nm/min. The final spectra were averaged over three scans, baseline corrected by subtracting the spectrum of appropriate blank solution and smoothed using a running average protocol available with the JASCO spectra analysis software.

Results and Discussion:

Expression of cytb5-tev-Mo1692: The gene encoding for peptide Mo1692 was cloned into pET21 vector. Mo1692 was expressed in fusion of cytochrome *b5* protein [11] with TEV cleavage site (cytb5-tev-Mo1692). The plasmids carrying the gene for fusion Mo1692 were transformed into over-expression vector BL21 (DE3, *E.coli*). Single colonies were picked from the plate and aseptically transferred onto LB-ampicillin broth. Overnight seed culture was grown in 20ml LB broth with 1% inoculum. Cultures were grown at 37°C and 180rpm in shaker-incubator until optical density of 0.6-0.8 at 600nm was attained. Protein was induced by the addition of IPTG to a final concentration of 0.5mM. The induction of the protein expression was observed by withdrawing 1ml of culture every hour, post induction. The cells were pelleted, lysed in Laemmli buffer and analyzed on SDS-PAGE gel electrophoresis (Fig. 1). Cells were then allowed to grow for a further period of 5hrs. Cells were harvested by centrifugation at 5000rpm at 4°C for 20 min. Supernatant was discarded and the cells were resuspended in TEG buffer (20mM Tris-HCl, 10mM EDTA, 50mM glucose, pH 8.0).

Protein purification: The cell suspension was lysed using a French press at a pressure of 1100psi. The cell debris was separated from the supernatant by centrifugation (12000rpm, 4°C, 30min). The cell lysate was loaded onto Sepharose-DEAE anion exchange column at 4°C. It was prequilibrated with 20mM Tris buffer (pH 8.0). The protein bound to column was washed with 5-6 column volumes of the same buffer to remove any unbound protein. Protein was eluted using 0-1M NaCl gradient. The eluted fractions were analyzed on SDS-PAGE gel (Fig. 2). The fractions containing protein were pooled and further purified by metal affinity chromatography using Ni^{2+}-IDA column. The column matrix was made up of imino diacetic acid groups and was charged with $NiSo_4$ prior to use. The column was equilibrated with 20mM Tris buffer (pH 8.0). In this purification step, apo form of protein binds to Ni^{2+} ion in the column and holo form comes out in the flow through during loading [11]. The protein was washed with buffer to remove any unbound protein. The protein was eluted with linear gradient of 0-1M imidazole. The purity of protein was checked on SDS-PAGE gel (Fig. 3). Pooled fractions were dialyzed against 20mM Tris buffer (pH 8.0). The protein was concentrated and subjected to gel filtration (FPLC) column for further purification. The protein was eluted at a retention volume of 176ml (Fig. 4). Higher level of purification was achieved by this chromatographic technique.

Characterization: The purified protein was characterized by UV–Vis spectroscopy, Mass spectrometry and Circular dichroism spectroscopy [13, 14]. The amount of protein was determined by measuring the absorbance at 280nm using calculated extinction coefficients [15]. Mass of this protein was confirmed by LC-ESI-MS and the deconvoluted mass of this protein is 13975Da (Fig. 5). CD spectrum of the fusion protein was recorded in the far-UV region of 190-260nm. The two bands at 208 and 222nm in spectrum showed the well folded structure of the fusion protein (Fig. 6).

Conclusion:

The successful expression and purification of Mo1692, a 15-residue marine snail venom peptide, was achieved through a systematic approach using bacterial expression and multi-step chromatographic purification. The cytb5-tev-Mo1692 fusion protein strategy proved effective for producing the recombinant peptide in E. coli BL21 (DE3) cells. IPTG induction at 0.5mM concentration successfully triggered protein expression, with optimal induction observed after 5 hours at 37°C, yielding a fusion protein of approximately 14kDa. The three-step purification protocol combining DEAE anion exchange, Ni^{2+}-IDA metal affinity chromatography and gel filtration chromatography achieved high purity levels. The final elution at 176ml retention volume on FPLC demonstrated effective separation and concentration of the target protein. Mass spectrometry confirmation (13,975Da) validated the correct molecular weight of the fusion protein. Circular dichroism spectroscopy revealed characteristic α-helical secondary structure features with bands at 208nm and 222nm, confirming proper protein folding and structural stability. The cytochrome b5 fusion tag system facilitated both expression enhancement and purification through metal affinity chromatography, while maintaining the structural integrity of the Mo1692 peptide domain [11].

This work establishes a robust protocol for producing Mo1692 peptide in recombinant form, providing a foundation for further structural and functional

characterization for potential therapeutic applications. The successful expression system can be scaled for larger production needs and may serve as a model for expressing other marine venom peptides with similar properties.

Acknowledgement

The author gratefully acknowledges Prof. Siddhartha P Sarma, Molecular Biophysics Unit, Indian Institute of Science, Bangalore for kindly providing the clone.

Funding

This work was supported by the grant from University Grants Commission, Govt. of India, New Delhi (No. F. 20-1/2012(BSR)/20-1(1)/2012(BSR) dated 26th June 2014) awarded to Dr. D. Zarena.

Conflict of interest

The author declares that there is no conflict of interest.

Ethics approval and consent to participate

This study did not involve any human subjects or animals. Therefore, ethics approval and consent to participate were not required.

***References**:*

1. Li R, Yu J, Ye D, Liu S, Zhang H, Lin H, Feng J & Deng K, Conotoxins: classification, prediction, and future directions in bioinformatics. *Toxins (Basel)*, 17 (2025) 78.
2. Olivera BM, Conus venom peptides: reflections from the biology of clades and species. *Annu Rev Ecol Syst*, 33 (2002) 25.
3. Terlau H & Olivera BM, Conus venoms: a rich source of novel ion channel-targeted peptides. *Physiol Rev*, 84 (2004) 41.
4. Robinson SD, Safavi-Hemami H, McIntosh LD, Purcell AW, Norton RS & Papenfuss AT, Diversity of conotoxin gene superfamilies in the venomous snail, *Conus victoriae*. *PLoS One*, 9 (2014) e87648.
5. Rockel D, Korn W & Kohn AJ, *Manual of the living Conidae* (Christa Hemmen Verlag, Wiesbaden) 1995.
6. Olivera BM, Conus venom peptides: reflections on the biology of a diverse group of bioactive molecules. *J Biol Chem*, 277 (2002) 34679.
7. Olivera BM, Conus venoms: a rich source of novel ion channel-targeted peptides. *Drug Dev Res*, 56 (2002) 173.
8. Morales Duque H, Campos Dias S & Franco OL, Structural and functional analyses of cone snail toxins. *Mar Drugs*, 17 (2019) 370.
9. Sudarslal S, Singaravadivelan G, Ramasamy P, Ananda K, Sarma SP, Sikdar SK, Krishnan KS & Balaram P, A novel 13 residue acyclic peptide from the marine snail, *Conus monile*, targets potassium channels. *Biochem Biophys Res Commun*, 317 (2004) 682.
10. Gowd KH, Sabareesh V, Sudarslal S, Iengar P, Franklin B, Fernando A, Dewan K, Ramaswami M, Sarma SP, Sikdar S, Balaram P & Krishnan KS, Novel peptides of therapeutic promise from Indian Conidae. *Ann N Y Acad Sci*, 1056 (2005) 462.
11. Mitra A, Chakrabarti KS, Shahul Hameed MS, Srinivas KV, Senthil Kumar G & Sarma SP, High level expression of peptides and proteins using cytochrome b5 as a fusion host. *Protein Expr Purif*, 41 (2005) 84.
12. Porath J, Carlsson J, Olsson I & Belfrage G, Metal chelate affinity chromatography, a new approach to protein fractionation. *Nature*, 258 (1975) 598.
13. Greenfield NJ, Using circular dichroism spectra to estimate protein secondary structure. *Nat Protoc*, 1 (2006) 2876.
14. Kelly SM, Jess TJ & Price NC, How to study proteins by circular dichroism. *Biochim Biophys Acta*, 1751 (2005) 119.
15. Pace CN, Vajdos F, Fee L, Grimsley G & Gray T, How to measure and predict the molar absorption coefficient of a protein. *Protein Sci*, 4 (1995) 2411.

Supplementary data

Fig 1: Induction profile of Mo1692 fusion protein

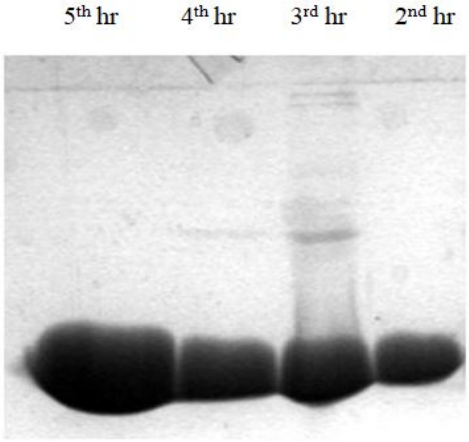

Fig. 3: Ni^{2+}-IDA eluted fractions

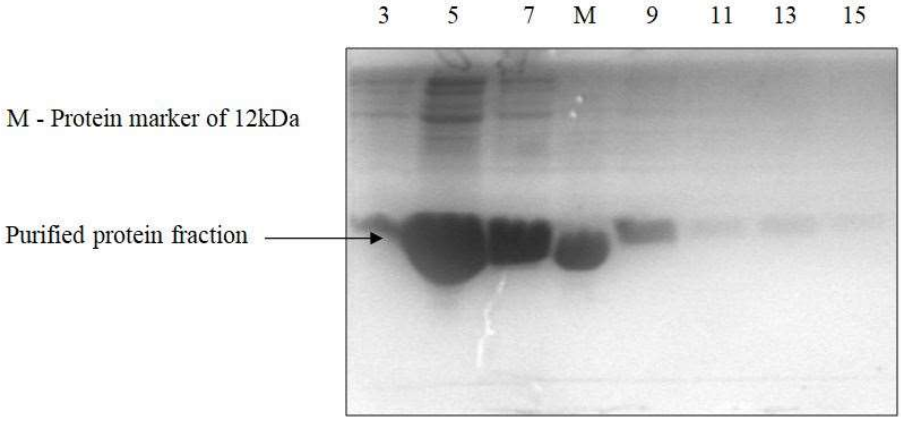

Fig. 5: LC-ESI-MS of Mo1692-cytb5 fusion protein

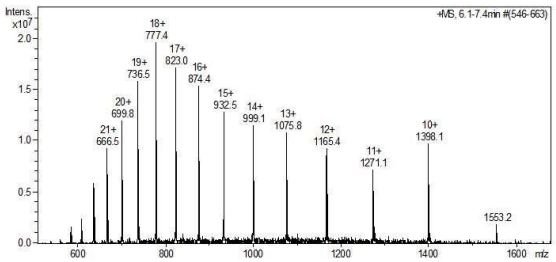

Fig. 2: The numbers above the lanes indicating the fractions of the fusion protein eluted from DEAE column

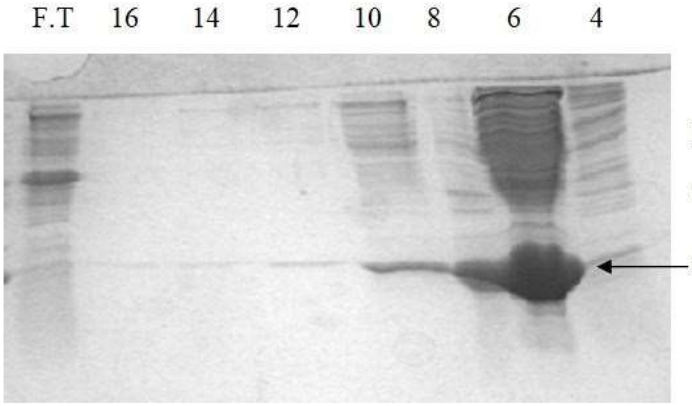

Fig. 4: Elution profile of Mo1692-cytb5 fusion on FPLC

Fig. 6: Circular dichroism spectrum of Mo1692-cytb5 fusion protein