Diversity of Conotoxins

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Conopeptides are small structurally diverse peptides secreted in the venom ducts of predatory marine cone snails. These neurotoxins are divided into two major groups, namely, the disulfide rich and disulfide poor. The former are termed conotoxins, which target a variety of ion channels, membrane receptors and transporters such as, voltage sensitive Ca^{2+} channels ($GABA_b$ mediated - N-type ($Ca_v2.2$) channels), voltage gated K^+ channels, voltage sensitive Na^+ channels, ligand gated ion channels (nicotinic acetylcholine receptors, nAChRs), and noradrenaline transporters. The cone snail belongs to the genus *Conus*, which is comprised of approximately 700 species that produce collectively up to 100,000 contopeptides. A single cone snail can secrete up to 200 different toxins. These gastropods use their repertoire of conotoxins with the purpose of capturing prey, defense, and competitive reasons.[1] Conotoxins have attracted attention in pharmacology due to the deadly consequences of human envenomation. Evolutionary adaptations and biotic interactions of the cone snail could have caused the production of vast variety of toxins in order to efficiently manipulate the nervous systems of at least five phyla of prey, the majority being fish, mollusk, and warms. Thus, the conotoxins produced by each snail is highly variable with little or no molecular overlap with peptides produced by another species.[2] The nomenclature and classification of conotoxins was originally employed by Cruz *et.al.* It groups the conotoxins into superfamilies based on sequence homology or cysteine arrangement in the primary sequence. It further subdivides them based on disulfide framework represented by a Roman numeral followed by a letter representing the order in which the conotoxin with the given framework was discovered. Finally the conotoxins are classified into pharmacological families denoted by a Greek letter based on their physiological targets.[3,4] Hence, conotoxin α-PnIA, shown in Figure 1, can be decoded as part of the A superfamily with I disulfide framework (Cys^{I}-Cys^{III} ; Cys^{II}-Cys^{IV}), which belongs to the α pharmacological family and it was the first to be discovered with I framework. As of today, there are over 2000 conopeptide sequences cataloged into the web-database ConoServer, which have helped the search and discovery of novel neuropharmaceuticals.[4] Conotoxins are initially translated by the ribosome into a canonical precursor peptide of about 100 amino acid residues, which contains three distinct regions encoded by individual exons.[5] The N-terminal signal sequence is approximately 20 amino acid residues and it is highly hydrophobic and homologous among a given superfamily. The next segment is a leader sequence, which is also conserved among superfamilies and it is between 20 to 60 amino acid residues. Some enzymes required for the

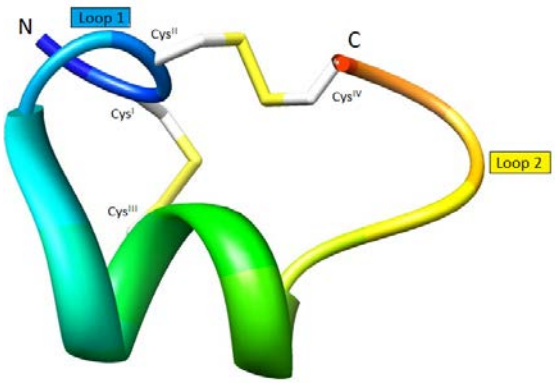
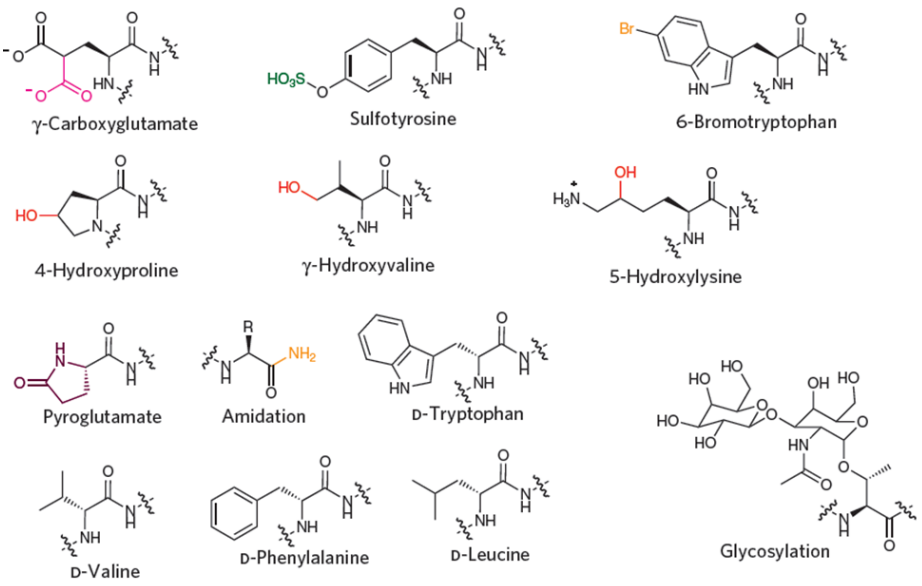


Figure 1: Crystal Structure of α-PnIA.

post-translational modifications bind to a recognition sequence found in the leader peptide. In some instances the leader peptide is found at the C-terminus following the mature core peptide and it is termed a post peptide. Finally, the mature toxin region is 11 to 30 amino acid residues and it is found at the C-terminus of the precursor peptide. In this region, hypermutations occur of the residues found between the highly conserved cysteine, which typically form loops that are essential for the selectivity and potency of a given receptor.[5-7]

Besides the disulfide formation mediated by protein disulfide isomerase (PDI)[8], the majority of conotoxins undergo a large amount of post-translational modifications in comparison to other secreted polypeptides.[9] Some common post-translational modifications essential for the maturation of the peptides are shown in Figure 2.[6,10] γ-Glutamyl carboxylase is a vitamin K dependent enzyme requiring the active oxygenated species of vitamin K alkoxide to abstract the γ-proton of glutamic acid with subsequent collapse to vitamin K epoxide catalyzed by vitamin K reductase. Carbon dioxide is added to the γ-glutamyl carbanion forming the product.[10,11] A γ-carboxylation recognition site (γ-CRS) has been identified in κ-TxX in the post peptide region with R-X-X-J-X-X-X-X-K/R motif, where X is any amino acid and J is any aliphatic hydrophobic residue usually Leu.[12] γ-Glutamyl carboxylase binds to the recognition sequence and tethers the substrate for carboxylation, as in the case of conantokins (one disulfide bond conopeptides) the sequence GEEE at the N-terminus of the mature toxin is typically modified to GEγγ, where γ stands for γ-carboxylated glutamate. [13,14] The carboxylation of glutamate was initially thought to be an innovation of the mammalian blood clotting cascade and later in tissue mineralization. Finding of γ-carboxy glutamate in the invertebrate system was of great surprise and showed that the post-translational modification could date back to millions of years before the division of invertebrates and vertebrates.[10,15] Prolyl hydroxylase has not been identified in conotoxins; however, the post-translational modification is frequently found in these peptides.[16] The hydroxylated proline is thought to assist in stabilization of the secondary structure in mammalian proteins. Prolines are typically hydroxylated in the P-Hyp-G repeats, where Hyp stands for 4-hydroxyproline. The catalytic mechanism for hydroxylation of proline is dependent upon a multi-subunit dioxygenase that requires Fe^{II}, 2-oxoglutarate, and O_2 to initially

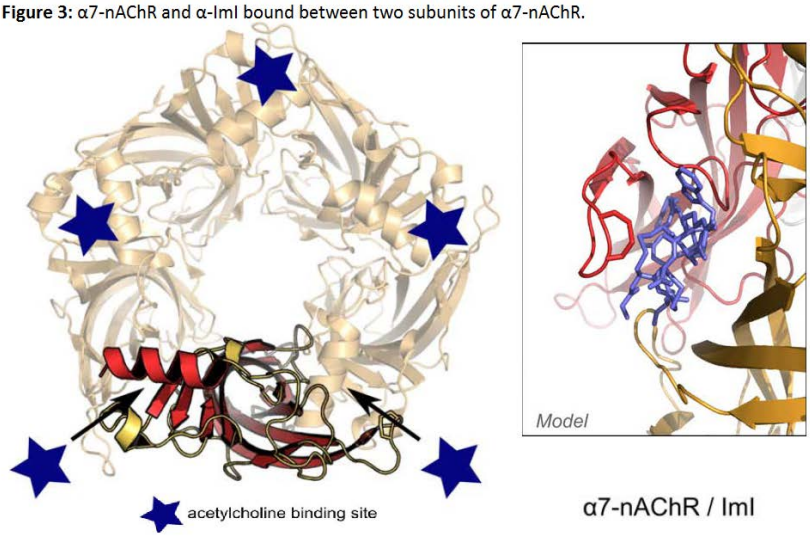
Figure 2: Post-translational Modifications in Conotoxins.



generate the highly reactive Fe^{IV} oxo-species that is responsible for hydroxylation of the substrate.[17] Other interesting post-translational modifications include sulfation of tyrosine, an adenosine 3'-phosphate 5'-phosphosulfate (PAPS) dependent reaction, which is responsible for the formation of the peptidyltyrosine O^4-sulfate ester and the bromination of tryptophan catalyzed by a bromo peroxidase.[18,19]

α-Conotoxins are competitive antagonists of cationic ligand-gated nicotinic acetylcholine receptors (nAChRs) found in the nervous system and muscular junctions in vertebrates. The loop size of this family of peptides defines the selectivity of each conotoxin to the corresponding receptor. The neuronal nAChRs are either homopetamers comprised only of α subunits, which trigger and regulate dopamine and norepinephrine release, or heteropentamers with a large assortment of α or β proteins.[20] The muscle nAChRs are in a ratio of two α_1, β, and γ (fetal type) or ε (adult type) subunits. Two acetylcholine molecules are required to bind to the receptor to initiate neurotransmission. The homopentameric structure of neuronal α-7 nAChR and the α-7 nAChR/α-ImI model complex is shown in Figure 3.[21] Conotoxins could act as non-competitive inhibitors by occlusion of ion conductance, or competitive antagonist by binding between specific subunits in both the fetal and adult type receptors. These conotoxins have been extensively studied as drug leads for the treatment of neuropathic pain due to degenerative diseases. [22]

Figure 3: α7-nAChR and α-ImI bound between two subunits of α7-nAChR.



The ω- conotoxin, MVIIA, has been approved for use in the United States and Europe for treatment of chronic pain; however, it has been presented with the challenge of being susceptible to degradation within the body. Other complications could occur due to the invasive intrathecal delivery of the drug. Thus, it is of interest to develop an orally active conotoxin with increased resistance to proteolytic degradation. Synthetic derivatives of the α-conotoxin Vc1.1 from the cone snail *Conus victoriae* has been cyclized and shown to be effective when administered orally. This 16 amino acid α-helical peptide contains an amidated C-terminus and Cys^{I}- Cys^{III} and Cys^{II}-Cys^{IV} disulfide bond arrangement. The cyclized derivative cVc1.1 exhibited higher potency in the concentration dependent inhibition of $GABA_b$ mediated N-type Ca^{2+} currents in rat dorsal root ganglion with IC_{50} of 0.3 nM in comparison to its linear Vc1.1 counterpart with IC_{50} of 1.7 nM. Further, the analgesic efficacy of the cyclized α-conotoxin was two orders of

magnitude higher than the current leading drug, gabapentin, for the treatment of neuropathic pain. The large pharmacological potential of conotoxins is due to the high selectivity exhibited on variety of neurological pain related receptors. Thus, conotoxins are excellent candidates for the development of orally active analgesics with minimal side effects and higher potency for the therapy of chronic pain.[23]

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