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Cyclization of conotoxins to improve their biopharmaceutical properties

Richard J. Clark, Muharrem Akcan, Quentin Kaas, Norelle L. Daly, David J. Craik*

The University of Queensland, Institute for Molecular Bioscience, Brisbane, QLD 4072, Australia

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ABSTRACT

Conotoxins are disulfide-rich peptides from the venoms of marine cone snails that are used in prey capture. Due to their exquisite potency and selectivity for different ion channels, receptors and transporters they have attracted much interest as leads in drug design. This article gives a brief background on conotoxins, describes their structures and highlights methods for synthetic cyclization to improve their biopharmaceutical properties. The proximity of the N and C termini of many conotoxins makes them particularly suitable for cyclization with linkers of on average five to seven amino acids. By linking the ends of conotoxins it is possible to significantly decrease their susceptibility to proteolysis without loss of their intrinsic biological activity. Here, the principles of conotoxin cyclization are illustrated with applications to the α- and χ- conotoxin classes, which have been implicated as leads for the treatment of pain and a range of other disorders including neuroprotection, schizophrenia, depression and cancer.

1. Introduction

Conopeptides are found in the venom of marine cone snails of the genus *Conus* and are used by these snails for prey capture (Gray et al., 1981; Olivera and Cruz, 2001). They include peptides that contain no disulfide bonds and those that are disulfide-rich, which are generally referred to as conotoxins (Olivera et al., 1991; Terlau and Olivera, 2004). Here we focus on the latter peptides. Different snail species within the *Conus* genus target different prey, including worms, fish and molluscs (Röckel et al., 1995). Clearly, to catch fast-moving prey, such as a fish, the components of the venom must be both fast-acting and potent so that, once envenomed, the fish cannot escape the slow-moving snail. This is indeed the case for conotoxins (Olivera, 1999, 2002; Olivera et al., 1999, 1985). Their exquisite potency has attracted attention as drug leads (Olivera et al., 2008) and thus why they are of interest to this special issue of Toxicon devoted to drug development.

Conopeptides have been studied extensively over the last 30 years (Gray et al., 1981; Kaas et al., 2010; Olivera and Cruz, 2001; Terlau and Olivera, 2004), and more than 200 conopeptides have been isolated and characterized, although it is estimated that more than 100,000 conopeptides exist (Han et al., 2008). Each snail expresses a suite of many conopeptides that together target a variety of receptors (Terlau and Olivera, 2004), with individual conopeptides being specific for particular targets. Indeed, conopeptides are categorized based on a number of criteria, with a main one being the receptor target. Table 1 summarizes the receptor targets for a small selection of conotoxins. The list is representative rather than exhaustive, and for sequence listings of all currently known conotoxins, readers are referred to ConoServer (http://www.conoserver.org), a web-based database and conopeptide resource site, where conopeptide sequences are updated regularly (Kaas et al., 2010; Kaas et al., 2008). Recent reviews (Kaas et al., 2010; Terlau and Olivera, 2004) describe conotoxin nomenclature in more detail but it suffices to say that conotoxins are generally divided into pharmacological families (denoted by Greek letters), reflecting the molecular targets, gene superfamilies (denoted by letters) based on conserved features of their gene sequences, and cysteine frameworks

* Corresponding author. Tel.: +61 7 33462019; fax: +61 7 33462029.
E-mail address: d.craik@imb.uq.edu.au (D.J. Craik).

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Table 1
Selected conotoxin sequences, origins, families and targets.

Toxin	Sequence[a]	Species	Superfamily/Family	Target
MII	G**CC**SNPV**C**HLEHSNL**C***	*C. magus*	A/α	nAChRs
MrIA	NGV**CC**GYKL**C**HO**C**	*C. marmoreus*	T/χ	norepinephrine transporter
MVIIA	**C**KGKGAK**C**SRLMYD**CC**TGS**C**RSGK**C***	*C. magus*	O1/ω	N-type calcium channels
PVIIA	**C**RIONQK**C**FQHLDD**CC**SRK**C**NRFNK**CV***	*C. purpurascens*	O1/κ	Potassium channels
GVIIIA	G**C**TRT**C**GGOK**C**TGT**CT**TNSSK**CGC**RYNVHPSGW^{Br}G**C**G**CA**S*	*C. geographus*	S/σ	5-HT_3 receptor

[a] * refers to amidated C-terminus; O is hydroxyproline and W^{Br} is brominated tryptophan Cysteine residues are highlighted in bold.

that describe cysteine patterns in primary sequence. The names of individual conotoxins comprise letters reflecting their species designator (e.g. G for *Conus geographus*), cysteine framework (denoted with Roman numerals), and order of discovery (denoted by a letter).

As mentioned above, conotoxins have exquisite selectivity for a diverse range of receptors, ion channels and transporters and thus they have sparked great interest in the pharmaceutical industry as leads in drug design (Adams et al., 1999; Halai and Craik, 2009; Livett et al., 2004; Olivera, 2006; Twede et al., 2009; Vincler and McIntosh, 2007). One conotoxin, ω-conotoxin MVIIA (also known as ziconotide) (Jain, 2000; Miljanich, 2004), is currently marketed under the trade name Prialt, for the treatment of chronic neuropathic pain. Other conotoxins have entered clinical trials; Xen2174 is in Phase II trials for neuropathic pain (Nielsen et al., 2005) and Vc1.1 (Satkunanathan et al., 2005) had also entered trials for neuropathic pain; however, this trial has since been halted. The remainder of this review will focus on two classes of conotoxins that are particularly prominent as drug leads, namely the α-conotoxins and the χ-conotoxins.

Although conotoxins have very promising therapeutic potential, there are generic limitations associated with most peptides, including susceptibility to degradation by proteases. One strategy for improving the stability of conotoxins, which we have adopted, is backbone cyclization (Armishaw et al., 2010; Clark et al., 2005, 2010; Lovelace et al., 2006). More broadly, cyclic peptides have been used in a variety of drug design applications in the pharmaceutical industry, and indeed cyclic peptides have been used clinically as drugs. The fungal product cyclosporin, for example, has revolutionized organ transplant therapy because of its potent immunosuppressant activities (Starzl, 1981). Cyclosporin is a 12 amino acid peptide that is non-ribosomally biosynthesized (Borel, 2002). Synthetic cyclization of designed peptides has also been used to lock the conformation or stabilize peptides (Zhou, 2004). However, so far, the synthetic or naturally occurring non-ribosomal cyclic peptides that have been applied in drug development have been smaller than ~12 amino acids in size. Most conotoxins are larger than this, with the size range of all conotoxins reported currently in ConoServer being 8–86 amino acids, and the average size being 26 (standard deviation of 10) amino acids (Kaas et al., 2010).

Furthermore, the examples of cyclic peptides that have been applied for drug design in the literature generally do not have disulfide bonds and hence are quite different to conotoxins. However, in unrelated studies over the last 15 years, a large number of naturally occurring disulfide-rich cyclic peptides have been discovered in plants and animals (Craik, 2006; Daly et al., 2009; Kawai and Saito, 2004; Selsted, 2004; Trabi and Craik, 2002). Key examples include the plant cyclotides (Craik et al., 1999), which are around 30 amino acids in size, sunflower trypsin inhibitor 1 (SFTI-1, 14 amino acids) (Luckett et al., 1999), and the mammalian theta-defensins, which are 18 amino acids in size (Cole et al., 2002; Selsted, 2004; Tang et al., 1999). The structures of these peptides are shown in Fig. 1. The common features of these naturally occurring cyclic peptides include exceptional stability and a compact structure, and together these make them potentially useful as templates in drug

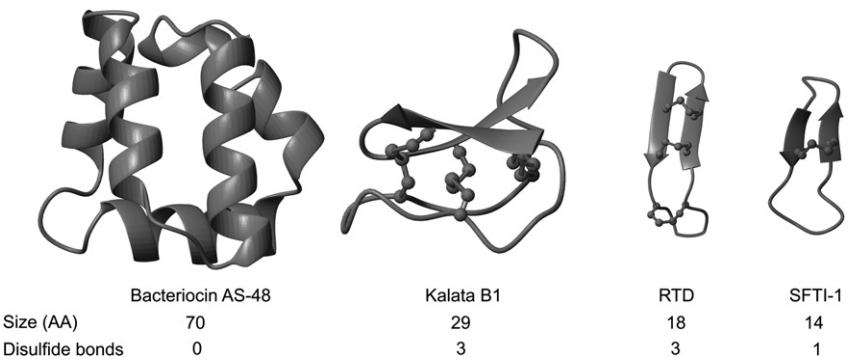

Fig. 1. Three-dimensional structures of selected naturally occurring cyclic peptides. AS-48 is a bacterial peptide with antimicrobial activity (Maqueda et al., 2004), kalata B1 a plant derived peptide with a range of bioactivities including insecticidal and anti-HIV activity (Rosengren et al., 2003), RTD-1 a mammalian peptide with antimicrobial activity (Trabi et al., 2001) and SFTI-1 a plant derived peptide with potent trypsin inhibitory activity (Korsinczky et al., 2005). Disulfide bonds are shown in ball and stick format. The β-sheets are shown as arrows and helices as ribbons.