

Future perspectives of venoms for drug discovery

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Abstract

Venoms have been regarded as an interesting source for screening difficult-to-address pharmaceutical targets like ion channels, transmembrane or circulating proteins for quite some time. Moreover, recent developments open up new opportunities to better exploit well-structured toxins - so called “mini-proteins” that we also like to refer to as “picologics” - in a more general way for lead discovery and drug development. In particular, progress in throughput and the first results from the next generation of genome/transcriptome analyses and systems biology investigations widen the scope of an integrated understanding of global transcriptional activity in venom glands. As a result, natural products from venoms are thought to represent a sustainable source of molecules for addressing a wider range of targets, and thus enable a more systematic approach to the identification of new peptide therapeutic candidates.

Animal venoms

Venoms typically consist of a cocktail of structurally and functionally different bioactives ranging from small molecules up to large proteins. However, most of the compounds fall within the class of well-structured medium sized peptides. These peptides are usually constrained by disulfide bridges, and therefore, adopt tertiary structures similar to those found in proteins. Although the venom of a given species can contain hundreds of such compounds (Figure 1), only about one hundred scaffolds are actually used by nature to convey high potency and selectivity towards proteins/receptors from different target classes. Each venom typically comprises 200-1'000 bioactives with members belonging to a few to dozen different scaffolds, sometimes with dozens of closely related analogues. However, this restricted number of scaffolds does not limit functional diversity. By contrast, these limited categories provide a foundation for a working hypothesis on the basis of a structure, since homology modelling enables construction of a plethora of molecules from a limited set of available data. Indeed, sometimes changing just one or a few amino acids in such a mini-protein can drastically change its potency and selectivity towards a pharmacological target without affecting its structural backbone.

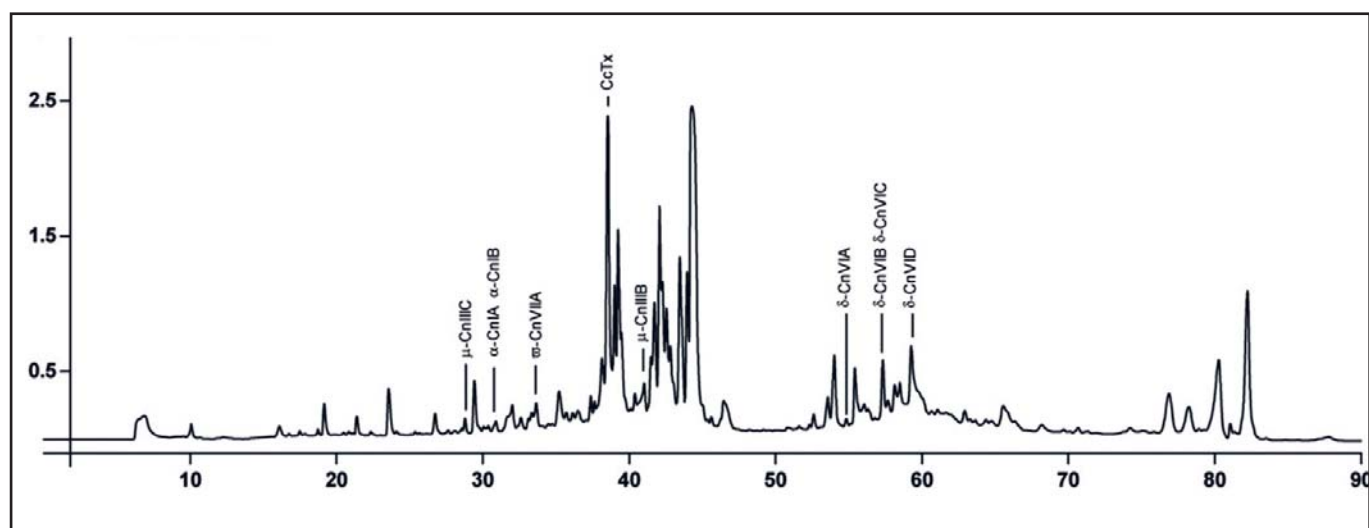


Figure 1: RP-HPLC chromatogram of the venom of the cone snail *Conus consors* in which more than 2'000 peptides and proteins have been detected to date by mass spectrometry in the frame of the CONCO project (www.conco.eu).

Venomomics

So far, venom proteomic, venom gland transcriptomic and genomic investigations have revealed taxon-specific trends for the formulation of venom complexity. Moreover, venom peptidomes provided qualitative and quantitative information over time on translation efficiency. This permits a better understanding of venom gland transcription as more data are being generated. As a result, a more accurate interpretation of the transcriptome, and more systematic studies in the field of venomomics – systems biology of the venomous function - become feasible. First results obtained in snakes and cone snails revealed a discrepancy between transcriptome-computed and proteome-based toxin compositions. In addition to other elements, interesting minor transcripts for cysteine-containing and cysteine-free peptides were observed enriching the diversity of potential bioactives. Another interesting aspect of venomomics studies relates to intra-species and even intra-specimen venom variations over time as has been described for the cone snail *Conus consors* (Figure 2). It is known that the composition of venoms from the same species can evolve over time depending on the conditions and external pressure encountered by the organism. From a more holistic perspective, the detection of enzymes, pore-forming proteins and cell-penetrating peptides helps in understanding how penetration of the venom and subsequent cytotoxicity for venom diffusion have evolved to efficiently capture and digest prey or to fight off predators.



Figure 2: The cone snail *Conus consors*.

Lead discovery

The standard preparation procedures for crude venoms require a pre-fractionation step prior to conducting bioassays to reduce sample complexity and to discard undesired effects that may be induced by cytotoxic components, pore forming toxins, proteases, hyaluronidases, nucleases or lipases. Once bioactives are identified in a primary screen, several rounds of sub-fractionation and re-screening can be required. Furthermore, the deconvolution process, to determine the complete primary structure of a highly potent hit present in a small sample, may require additional material. Considering the real world, the access to a sufficient amount of venoms, *e.g.* for screening in the frame of lead discovery campaigns, often remains a challenge, in particular for smaller organisms and/or rare species (Figure 3). Hundreds of thousands of venomous species originating from all major phyla can be found on earth, in the air and underwater. Amongst those, the venom of only a few hundred have been made commercially available to the scientific community, mainly from snakes and a few easily accessible scorpions, spiders, amphibians and hymenoptera. To date, the structure of approximately 10'000 venom components has been elucidated, mostly from cDNA libraries. This represents maybe 1/1'000 of the molecular biodiversity offered by these creatures, only a portion of which has undergone functional studies on a limited number of pharmacological targets. As such, the five venom-derived drugs that are currently on the market represent only the very tip of an iceberg that still has many promises to deliver.



Figure 3: Small organisms like a caterpillar, a spider or bee harbour only tiny amounts of venom.

Next generation sequencing

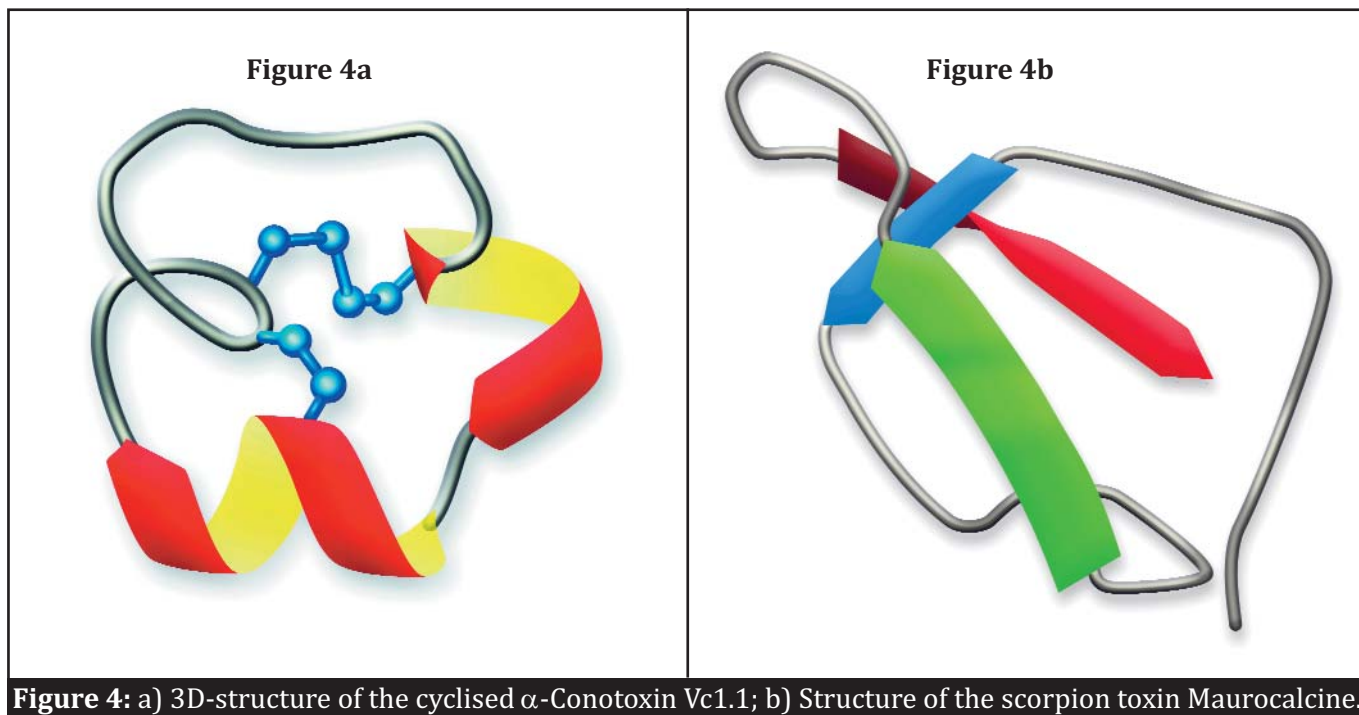
With exponentially increasing capacities and decreasing costs, high-throughput next generation (Nex-Gen) sequencing strategies open up the door for overcoming the hurdle regarding the availability in limited amounts of venoms from strikingly small or extremely rare species. As these studies progress and the genome/transcriptome of more species become available, the deconvolution process is also significantly facilitated. Following identification of a high-resolution mass and/or partial sequence information (MS/MS or Edman for example) for a single active peptide, a match to the appropriate databases provides immediate access to the structure, thus avoiding time-consuming experiments. Furthermore, dedicated bioinformatic tools allow for the identification of analogues of hits, both in the same and in other transcriptomes, generating useful lists of structurally-related compounds. This provides useful clues as to which amino acid positions have been modified under evolutionary pressure to modulate the bioactivity of venom components, which serves as a basis for structure-function studies and lead optimization programs. In the long run, massive *de novo* venom gland transcriptome sequencing and targeted genomic studies will facilitate the identification of new picologics, including the discovery of novel scaffolds, which will catalyse *in silico* virtual screening initiatives using innovative bioinformatics platforms. In addition, these efforts will pave the way to the construction of synthetic libraries enabling fast, cheap, ethical, and safe access to these interesting classes of natural products. In principle, all putative bioactive compounds of a species of interest then become available, and thus, offer a sustainable source of pure compounds of natural origin for screening.

Drugability

Linear peptides are usually rapidly degraded in plasma. In the case of venoms, nature has already optimized bioactives for a certain stability, since most of the organisms store the venom cocktail at ambient temperature for days up to months in the presence of numerous potent proteases. As a result of their small compact size, venom components are also known to be poorly immunogenic. In addition, venoms are highly concentrated biofluids, and therefore, their components are most often highly soluble. Most interestingly, however, is the strategy selected through million years of molecular evolution by which venoms are not made of one single component with universal toxicity that would irremediably affect any prey or predator. Instead, to prevent the risk of resistant organisms and to protect the animal from its own venom, venoms have evolved as highly complex mixtures made of hundreds of highly potent bioactives, each selective for one specific target, the expression of which might be modulated over time depending on the animal's needs. Accordingly, the medicinal chemist's work is facilitated and, in general, fine-tuning of the molecules focuses on optimisation of activity, selectivity and delivery. Indeed, screening of venom libraries typically provides sub-micromolar primary hits coupled to high selectivity, and thus, in general, an activity of interest does not necessarily need to be improved by several log-units. This comfortable situation and a working-hypothesis based on structural data allow to efficiently convey most drugability requirements into the peptide of interest, named picologics by contrast to macromolecular biologics. Thus, bearing in mind that most venom-derived picologics are simple to synthesize and knowing that cost of goods is not an issue, the major problem remains in most cases to achieve appropriate delivery.

Delivery

Progress in this direction was reported in 2010 for the α -Conotoxin Vc1.1. Following a backbone cyclization in addition to the 2 Cys-bridges, the molecule changed selectivity and became orally active (Figure 4a). Since only a very limited number of analogues were actually synthesized, it seems there is additional room for optimization and in the long term it may well be feasible to achieve oral delivery for a large number of peptide drugs. In another unrelated study, intracellular transport for the scorpion toxin Maurocalcine and its D-analogue have been demonstrated (Figure 4b). Whilst this toxin possesses some of the principle features of cell penetrating peptides (CPPs), the molecular mechanisms, in particular the selectivity observed under certain conditions, the interaction of the toxin with the cell membrane, and the translocation event have not yet been elucidated. However, this observation clearly shows the potential of these well-structured mini-proteins to overcome delivery hurdles if the relevant features can be designed into an appropriate scaffold.



Outlook

Looking ahead, the various activities in venomics, in particular at the genomic, transcriptomic and proteomic levels, will provide a more comprehensive knowledge of the molecules present in venom cocktails and a better understanding of the synergistic effects achieved by evolution. These studies will also support a more sustainable production for this class of natural compounds, thereby preserving rare species for the future. The recent initiatives regarding genomic and transcriptomic data mining will also speed-up the deconvolution process, which is in general regarded as time-consuming.

In spite of this current complication, recent developments in the quest for the discovery of innovative leads have focused on the exploration of natural libraries. These activities include the large-scale preparation of pre-fractionated venoms ready-made for high-throughput screening (www.melusine.com). Achievements on the analytical side, in particular in mass spectrometry and Next-Gen sequencing, improve the situation with respect to deconvolution. Bioinformatics-assisted strategies have also now proven their ability to deliver beyond expectations and clearly deserve further development efforts. In addition, state-of-the-art chemical peptide synthesis and recombinant strategies now address complex structures and can easily be applied in order to fine-tune properties. Further down the road, efficient large-scale manufacturing of mini-proteins has already been realized, and as a consequence, there are no significant issues on the manufacturing side regarding their development and commercialisation. For these reasons, venom components, or generally speaking mini-proteins, benefit from the advantages of both small molecules and biologics whilst suffering much less from their drawbacks, although oral bioavailability remains a challenge. These “picologics” are likely to become an important class of innovative therapeutics in the future, bridging the gap between small molecules and biologics.

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