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Toxic polypeptides of the hydra—a bioinformatic approach to cnidarian allomones

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Abstract

Cnidarians such as hydrae and sea anemones are sessile, predatory, soft bodied animals which depend on offensive and defensive allomones for prey capture and survival. These allomones are distributed throughout the entire organism both in specialized stinging cells (nematocytes) and in the body tissues. The cnidarian allomonal system is composed of neurotoxins, cytolysins and toxic phospholipapses.

The present bioinformatic survey was motivated by the fact that while hydrae are the most studied model cnidarian, little is known about their allomones. A large-scale EST database from *Hydra magnipapillata* was searched for orthologs of known cnidarian allomones, as well as for allomones found in other venomous organisms.

We show that the hydrae express orthologs of cnidarian phospholipase A2 toxins and cytolysins belonging to the actinoporin family, but could not find orthologs of the 'classic' short chain neurotoxins affecting sodium and potassium conductance. Hydrae also express proteins similar to elapid-like phospholipases, CRISP proteins, Prokineticin-like polypeptides and toxic deoxyribonucleases. Our results illustrate a high level of complexity in the hydra allomonal system, suggest that several toxins represent a basal component of all cnidarian allomones, and raise the intriguing possibility that similar proteins may fulfill both endogenous and allomonal roles in cnidaria.

Keywords: Hydra; Allomone; Venom; Nematocyst; Phospholipase; Actinoporin; Neurotoxin; Prokineticin; CRISP

1. Introduction

Various interactions between organisms are mediated by chemistry. These interactions can be classified according to their ecological role into communication between members of the same species (pheromones), communication between members of different species (kairomones), and offensive or defensive chemicals (allomones) (Burks and Lodge, 2002; Ruther et al., 2002). In this context, offensive allomones are substances employed by a predatory organisms in order to paralyze and subdue their prey, while defensive allomones are substances employed in order to deter enemies (predators or territorial competitors). Allomones which are produced and stored in a localized glandular tissue and delivered through a piercing delivery device comprise a venom apparatus (Edstrom, 1992).

Sessile cnidarians such as sea anemones and hydrae are extremely dependent on allomones. Firstly, as static predators, which feed on mobile prey organisms, they depend on offensive allomones to paralyze and subdue their prey. Secondly, as exposed, soft-bodied motionless creatures, they rely on chemistry to protect them from predators, from territorial competitors, from fouling organisms and

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from pathogenic bacteria. However, cnidaria do not possess a defined venom apparatus in the common conventional sense. Firstly, the cnidarian allomonal system is distributed throughout the animals' body, including not only the hunting tentacles but also other organs such as the body column, specialized aggression organs such as acrorhagi, and gastrodermally derived tissue such as acontia and mesenterial filaments ((Hessinger and Lenhoff, 1973) and our unpublished results). Secondly, toxic chemistry can be found in both the elaborate stinging cells (nematocytes) (Tardent, 1995) and in the non-nematocystic tissue (Zhang et al., 2003). The chemical arsenal of cnidarians is also diverse, comprised mainly of polypeptides, and includes neuroactive ion channel modifiers, enzymes, and various pore-forming cytoltic proteins (Anderluh and Macek, 2002; Gasparini et al., 2004; Lotan et al., 1996; Norton, 1991). In addition, non-protein substances can also be found (Gleibs and Mebs, 1999). In all of these regards cnidarians differ from other venomous organisms in that they function as a 'holistic' allomonal system which uses diverse organs, modes of delivery and functionally diverse toxic substances. The above chemical and pharmacological sophistication is in contrast to the simple-primitive morphology and anatomy of these animals (Hickman, 1988).

Hydrae are tiny freshwater cnidarians belonging to the order hydrozoa, which also contains highly venomous animals such as the fire coral *Millepora* and the Portugese Man-O-War *Physalia*. Similar to larger cnidarians, hydrae are predators, feeding on small arthropods and larval fish, which they catch using the nematocytes on their hunting tentacles. (Elliott et al., 1997; Haynes, 1973). In contrast to the extensive work invested into studying the chemistry and pharmacology of marine cnidarians (reviewed by Anderluh and Macek, 2002; Norton, 1991), little toxinological work has been devoted to studying toxins from hydrae (Klug and Weber, 1991; Klug et al., 1989; Lesh-Laurie et al., 1989; Weber et al., 1987, 1988; Zhang et al., 2003), with only one non-nematocystic toxin characterized to the level of the primary structure (Zhang et al., 2003). On the other hand, hydrae have been studied extensively for over 200 years, and are the first cnidarians for which basic molecular methodology is available (Bosch et al., 2002; Lohmann et al., 1999; Miljkovic et al., 2002).

Recently, a large-scale EST sequencing effort from *Hydra magnipapillata* has been initiated in order to gain an understanding of the hydra gene set. The result of this collaborative effort is an extensive EST database (with over 100,000 ESTs sequenced corresponding to ~16,000 distinct sequences representing ~13,000 different genes) which is available online at www.hydrabase.org. We used a bioinformatic approach to provide an overview on cnidarian allomones, and utilized this EST database in order to search for components of the hydras' allomonal system. We show that this primitive cnidarian expresses several, but not all, of the known cnidarian toxins, as well as orthologs of highly specific and elaborate reptilian, arachnid and molluscan venom components. These may fulfill dual, allomonal as well as endogenous, roles in the biology of cnidarians.

2. Bioinformatic methods

2.1. General

The sequence information used for this work was generated as part of the Hydra EST Project (www.hydrabase.org). The sequencing for the Project was carried out at the Genome Sequencing Center at Washington University (http://genome.wustl.edu/). All the bioinformatic work presented was carried out from May to August of 2004, and represents the sequence information available at that time.

2.2. Compiling an overview of known cnidarian toxins

To search for hydra toxins, we first compiled a list of all the toxins from cnidaria whose amino acid sequence has been determined and deposited in the NCBI protein database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Protein). We did this by searching the non-redundant (NR) database at the NCBI for 'txid6073[Orga-txid6073[Organism:exp] and toxin', resulting in 108 sequences. Preliminary grouping was achieved by using CLUSTALW at EBI to create a 'phylogenetic tree', which was then used as a basis for manual sorting of the sequences into groups based on similar biological activity and/or sequence similarity. In addition, redundant sequences, or partial sequences of proteins whose full sequence had later been determined, were removed, resulting in 58 distinct toxin sequences (Table 1).

2.3. Searching the hydra EST database for orthologs of known cnidarian toxins

Utilizing the BLAST tool (Altschul et al., 1990) found in the Hydra EST Project database, we searched the ESTs for the 'archetype' sequences found in Table 1. Each search was performed twice, once using the default settings (suitable for relatively long segments of sequence similarity) and again using different settings aimed at better identification of short, nearly exact sequences (PAM30 matrix instead of BLOSUM62, expect 1000 and no filtering of low complexity regions, http://www.ncbi.nlm.nih.gov/BLAST/producttable.shtml#shortp). While many putative orthologs were found, only those with an e-value of above 0.5 were chosen for further characterization.

2.4. Sequence analyses

The sequences chosen for further characterization, whose accession numbers are provided in Tables 1 and 2, were translated using the translation tool found in the ExPASy website (http://www.expasy.org), in order to