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Structures of sea anemone toxins

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ABSTRACT

Sea anemones produce a variety of toxic peptides and proteins, including many ion channel blockers and modulators, as well as potent cytolysins. This review describes the structures that have been determined to date for the major classes of peptide and protein toxins. In addition, established and emerging methods for structure determination are summarized and the prospects for modelling newly described toxins are evaluated. In common with most other classes of proteins, toxins display conformational flexibility which may play a role in receptor binding and function. The prospects for obtaining atomic resolution structures of toxins bound to their receptors are also discussed.

1. Introduction

Sea anemones possess numerous tentacles containing specialized stinging cells or cnidocytes, which are in turn equipped with organelles known as nematocysts that contain small threads which are everted forcefully following mechanical or chemical stimulation. Anemones use this venom apparatus mainly in the capture of prey (crustaceans, small fish) and for defence against predators, although possibly not for intraspecific aggression (Bartosz et al., 2008). In keeping with other venomous animals, sea anemones contain a variety of interesting biologically active compounds, including some very potent toxins (Béress, 1982).

Peptides and proteins figure prominently amongst the sea anemone toxins characterized to date (Béress, 1982; Norton, 1991; Honma and Shiomi, 2006; Norton, 2006). The two most thoroughly characterized classes are the 5 kDa toxins that act by binding to the voltage-gated sodium channel and the 18–20 kDa cytolysins. This review focuses on the structures and structure–function relationships of these and other sea anemone toxins. Three-dimensional structures have been determined for other classes of sea anemone proteins, notably a range of fluorescent proteins that emit light in the far-red, red, yellow, cyan and blue regions of the visible spectrum (Chan et al., 2006), but these have no known toxic function and will not be discussed in this article. Protease inhibitors will be considered briefly because the Kunitz-type fold adopted by some of these proteins has assumed ion channel inhibitory functions in sea anemones (Schweitz et al., 1995; Honma et al., 2008) and probably also in the closely related cnidarian *Hydra magnipapillata* (Sher et al., 2005).

We begin with a brief description of biophysical methods available for studying peptide and protein structure and interactions. The structures of those toxins for which high-resolution structures have been determined are then described. Finally, we discuss the functional implications of these structures and the challenges remaining to defining the molecular basis for their various activities.

Abbreviations: AP-A, anthopleurin-A; AP-B, anthopleurin-B; CD, circular dichroism; EqtII, equinatoxin II; HERG, human *ether-a-go-go*-related gene; Kv, voltage-gated K^+ channel; K_{Ca}, Ca^{2+}-activated K^+ channel; PC, phosphatidylcholine; ShK, K^+ channel toxin from *Stichodactyla helianthus*; SM, sphingomyelin; VGSC, voltage-gated sodium channels.

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2. Structural methods

2.1. Spectroscopy

Various biophysical methods can provide low-resolution structural information more quickly and often utilizing much less protein than the higher resolution methods described below. Circular dichroism (CD) spectroscopy is a very powerful tool for characterizing protein secondary structure and detecting conformational changes over a range of conditions, which might include changes in pH, temperature and solvent or the presence of a binding partner (Kelly et al., 2005). While CD spectroscopic analysis of peptides and proteins in aqueous solution is relatively straightforward, studies in lipid vesicle systems are more challenging because of differential light scattering effects caused by the vesicles. Moreover, the high lipid:protein ratios usually required to mimic physiological conditions result in low protein concentrations and thus low intensity spectra. Synchrotron radiation CD (SRCD) spectroscopy is able to overcome these problems, and has the advantage that spectra can be recorded to lower wavelengths (around 175 nm instead of 190 nm), thereby providing more accurate secondary structure information (Miles and Wallace, 2006). As an example, SRCD has been used recently to examine membrane interactions of the pore-forming actinoporin equinatoxin II (EqtII) (Miles et al., 2008).

Fourier transform infrared (FTIR) spectroscopy can also be used to monitor secondary structure, and is applicable to proteins bound to membranes (Barth, 2007). A recent study of the interaction of the actinoporin sticholysin II with membranes (Alegre-Cebollada et al., 2007) demonstrates that valuable information can be obtained by this approach, although overlap between protein and lipid signals can be problematic. Another drawback of infrared spectroscopy studies of proteins in aqueous solution is the strong absorbance of water in the mid-infrared spectral region (near 1645 cm^{-1}), which overlaps with several important protein backbone and side chain absorbance bands. This requires the use of short path lengths (around 5 μm) and high protein concentrations for aqueous samples. To overcome this problem, samples can be prepared in ^2H_2O since its absorbance band is shifted to ~1210 cm^{-1}. Another form of vibrational spectroscopy which provides essentially the same information as FTIR but can be used in aqueous solution is Raman spectroscopy (Barth, 2007). This method has not been used extensively in studies of toxins but was applied in one of the early studies of sea anemone toxin conformation to assess secondary structure content and monitor side chain environments (Prescott et al., 1976). Raman optical activity, which is measured as a small circularly polarized component in Raman-scattered light from chiral molecules, is a promising extension of conventional Raman spectroscopy for studying proteins in aqueous solution (Barron, 2006) and may be useful in future studies of toxin structure and interactions.

Fluorescence spectroscopy is a potentially valuable technique for probing the environment of residues that contribute to the observable signal, but suffers from the limitation that only a few residue types fluoresce and even these may be quenched in a native protein. The information available from fluorescence studies of toxins bound to membranes has been reviewed recently using the cholesterol-dependent cytolysins from bacteria as one example (Johnson, 2005), and several applications to sea anemone cytolysins have been reported (e.g. Alvarez et al., 2001; Gutierrez-Aguirre et al., 2004). Fluorescence energy transfer is another powerful tool for studying molecular interactions and conformational changes, and single-molecule detection techniques promise to extend the reach of this and other fluorescence methods to unprecedented spatial resolution (Haustein and Schwille, 2004).

2.2. Atomic resolution structures

The principal methods employed for the determination of atomic resolution structures of peptides and proteins are nuclear magnetic resonance spectroscopy and X-ray crystallography. Of these two methods, NMR spectroscopy has been used more widely for solving sea anemone toxin structures. NMR can be applied without isotopic labelling to small proteins up to 6–7 kDa in mass (Wuthrich, 1986; Hinds and Norton, 1994) although the exact limit depends on the resolution of the ^1H NMR spectrum and the magnetic field strength being used (generally speaking, the higher the better). Fortunately, a significant number of interesting toxins, not just from sea anemones but also other venomous creatures, fall within this mass range. For larger proteins, isotopic labelling is required (Yokoyama, 2003; Foster et al., 2007). Up to 10 kDa, ^{15}N labelling coupled with a few straightforward three-dimensional NMR experiments should produce a good quality structure, but beyond this point double labelling with ^{15}N and ^{13}C is required. Isotopic labelling requires that the protein be expressed and refolded in a heterologous expression system that can grow on minimal media supplemented with ^{15}N- and ^{13}C-labelled sources of nitrogen and carbon, respectively. Bacterial systems are usually preferred, but the over-expressed toxin proteins often form inclusion bodies, in which case it is necessary to solubilize the expressed protein under denaturing and reducing conditions and then refold the protein *in vitro* (Gallagher and Blumenthal, 1992). Alternatively, folded toxins can be obtained by bacterial expression of soluble fusion proteins from which they are released by protease cleavage (Moran et al., 2006, 2007; Stehling et al., 2008). Yeast offers the advantage that the desired protein can be secreted with native disulfide bonds already formed, while insect cells are used much less often, but they can support isotopic labelling (Strauss et al., 2005).

The enormous investments made in several countries in structural genomics/proteomics have benefitted all stages of the structure determination pipeline, whether by NMR or X-ray crystallography (Gileadi et al., 2007; Phillips et al., 2007). Methods for producing proteins, assessing whether they are suitable candidates for structure determination, collecting and analyzing the data, and evaluating the final structures obtained have all undergone significant improvements, which have in turn flowed back to the entire structural biology community, including those interested in toxins. It has been argued that structural genomics has less to offer toxinology because many potent toxins have similar folds but quite distinct biological