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REVIEW

Crustins: Enigmatic WAP domain-containing antibacterial proteins from crustaceans

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Summary
Crustins are antibacterial proteins of ca. 7–14 kDa with a characteristic four-disulphide core-containing whey acidic protein (WAP) domain, expressed by the circulating haemocytes of crustaceans. Over 50 crustin sequences have been now reported from a variety of decapods, including crabs, lobsters, shrimp and crayfish. Three main types seem to occur but all possess a signal sequence at the amino terminus and a WAP domain at the carboxyl end. Differences between types lie in the structure of the central region. Those crustins purified as the native protein or expressed recombinantly all kill Gram-positive bacteria, and gene studies have shown that they are constitutively expressed, often at high levels, but show no consistent patterns of change in expression following injection of bacteria. This variable response to infection is enigmatic but indicates that these proteins could perform additional functions, perhaps as immune regulators in recovery from wounding, trauma or physiological stress.

Contents

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Introduction

The Crustacea is the largest, most conspicuous and, arguably, the most important group of marine or aquatic arthropods in terms of their biomass and ecological or economic value. Crustaceans have been popular experimental animals in nearly all aspects of biology, but it is decapods that attract most attention in relation to their immune responses because of their huge commercial importance and the need to control disease outbreaks in shellfish aquaculture. Given the ecological and economic significance of crustaceans and the amount of knowledge that has been gained about their defence responses, it is very surprising that it has taken so long for a body of work to build up on the presence of the low molecular weight defence effectors, more popularly known as antimicrobial peptides (AMPs), in these animals. To date, over 800 AMPs from eukaryotic organisms have now been reported in the literature or lodged on databases (see, for example, http://www.bbcm.univ.trieste.it/tossi/pag1.html, http://public-1.cryst.bbk.ac.uk/peptaibol/home.shtml, http://research.i2r.a-star.edu.sg/Templar/DB/ANTIMIC/), yet the majority are still derived from mammals, insects or amphibians. So far less than 10% of all known animal AMPs are from crustaceans, with nearly all of these found in decapods.

Whilst numerous papers have described antimicrobial activities of crustacean blood, tissues or body fluids (see for example [1–3]), it was not until 1995 that the first crustacean AMP was isolated and characterised. This was a 6.5 kDa proline-rich cationic peptide, purified from the haemocytes of the shore crab, *Carcinus maenas*, with activity against both Gram-positive and Gram-negative bacteria [4]. Soon after, reports began to emerge of antibacterial proteins from other species, notably those from shrimp [5]. The number is now rising steadily with over 70 from a variety of species presently listed on databases or described in the literature. They comprise a diverse collection of proteins with two main groups: the penaeidins and crustins. The penaeidins are cationic AMPs of 5–7 kDa, characterised by a proline-rich amino-terminal domain and a cysteine-rich carboxyl terminus domain. They seem to be confined to the Dendrobranchiata and have been reviewed elsewhere [6–8]. The crustins, on the other hand, are ca. 7–14 kDa, cysteine-rich and occur more widely across the decapoda, having been found in both Pleocyemata and Dendrobranchiata.

The first crustin to be found was an 11.5 kDa cationic and hydrophobic protein isolated from the haemocytes of the shore crab, *C. maenas* [9]. This protein was later designated carcinin [10] and judged to be a crustin-type molecule following the determination of its full coding cDNA and its recombinant expression *in vitro* [11]. Carcinin remains the only crustin to have been isolated in its native form from crustacean haemolymph, cloned and expressed recombinantly *in vitro*. The name 'crustin' was originally invoked to describe a cys-rich gene, with high sequence homology to carcinin, in the shrimp, *Litopenaeus vannamei* [12]. However, many more of these cys-rich carcinin-like genes have been subsequently detected in crustaceans and it is becoming clear that they are expressed by many, making the term more generic. To date some 50, including isoforms and ESTs, have been found in 20 crustacean species including crabs, lobsters, shrimp, prawns and crayfish (Table 1). As crustins are relative newcomers to the abecedary of invertebrate immune molecules, there is some confusion as to what constitutes a member of the crustin group, what biological effects they have and how they respond to non-self-challenge. Accordingly, the present review is aimed at bringing together the available data on this group of proteins, summarising their characteristics and diversity and discussing their biological roles.

Definition of a crustin

There is no existing universally accepted definition of a crustin, but the present review considers them to be cationic cysteine-rich antibacterial polypeptides of ca. 7–14 kDa, with an isoelectric point usually in the range of 7.0–8.7, present in crustaceans that contain one whey acidic protein (WAP) domain at the carboxyl terminus. This domain has eight cysteine residues in a conserved arrangement that forms a tightly packed structure described on PROSITE as a four-disulphide core (4DSC). The terms 'WAP domain' and '4DSC' are now coming to be used synonymously and this review does not make a distinction between them.

The 4DSC motif is not unique to crustins. The term 'WAP' is derived from the name given to a family of proteins, originally discovered in the whey fraction of mammalian milk. Whilst all these milk proteins are characterised by possession of two WAP domains, each comprising 50 amino acids (aa) [13], numerous other non-milk WAPs are now also known and these may have one or more 4DSCs. Amongst these non-milk WAPs are small secretory proteins with protease inhibitory properties or regulatory functions in growth, tissue differentiation or regulation and may sometimes be expressed in certain cancer states [14,15]. A few are antibacterial [16,17]. Particularly well-known WAP domain-containing proteins in mammals are antileukoproteinases, elafins and trappins. Analysis of numerous WAPs from vertebrates reveals a high degree of similarity between the WAP domain structures, and Ranganathan et al. [13] have proposed that the PROSITE definition of the domain structure