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Antimicrobial Peptides in Fiddler Crabs: Structural Analysis and Potential Applications

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Abstract

Crustaceans represent the largest and most ecologically and economically significant group of marine and aquatic arthropods. Their biomass and critical role in ecosystems highlight their importance. Among crustaceans, decapods are frequently used as model organisms in studies of immune responses due to their significant commercial value and the need to mitigate disease outbreaks in shellfish aquaculture. Antimicrobial host-defense peptides (AMPs) are pivotal in metazoan immunity, especially for invertebrates lacking adaptive immune systems. These peptides exhibit potent neutralizing actions against various pathogens, including Gram-positive and Gram-negative bacteria, yeast, fungi, and enveloped viruses. Beyond their antimicrobial functions, AMPs also regulate immune components during host-pathogen interactions. This study focuses on crustin, an antimicrobial protein from fiddler crabs, with sequences obtained in FASTA format from the NCBI database. Structural and functional analyses predict wide-ranging medical applications. The modeled protein structures were verified using Procheck software.

Keywords: Crustacea, antimicrobial peptides, fiddler crab, Crustin, bioinformatics, NCBI, protein modeling

INTRODUCTION

Fiddler crabs are fascinating intertidal species that thrive in a range of coastal habitats, including mangrove forests, expansive mudflats, sandy shores, and occasionally rocky beaches. These crabs often form dense populations, with each individual occupying a distinct burrow. These burrows are surrounded by small patches of surface sediment and serve critical functions such as protection against predators, maintaining moisture for gill function, and providing safe spaces for reproductive activities, including mating and egg incubation during low tide. Additionally, during high tide, the burrows act as secure refuges for these crabs.

Ecologically, fiddler crabs play a vital role in maintaining the health of intertidal ecosystems. Their feeding and burrowing activities contribute to nutrient cycling, sediment aeration, and habitat maintenance. As prey for a variety of predators, fiddler crabs occupy an integral position within coastal food webs. Furthermore, their bioturbation activities, which involve reworking sediments, influence the physical and chemical properties of their environment, highlighting their ecological importance [1].

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The feeding habits of fiddler crabs are uniquely adapted and differ between sexes. Female fiddler crabs have two small, equally sized feeding claws that allow them to gather food more efficiently. In contrast, males possess one large claw, primarily used for display and competition, which reduces their feeding efficiency. Both sexes collect sediment using their smaller claws, filtering organic material

through specialized mouthparts. Once the organic content is extracted, the cleaned sediment is expelled in the form of small sand pellets, which accumulate across the mudflat surface by the end of the low tide cycle.

In crustaceans, antimicrobial peptides (AMPs) serve as a crucial component of innate immunity, providing a primary line of defense against microbial threats. AMPs are globally recognized for their role as natural antibiotics and have garnered significant interest in their potential applications in combating antibiotic resistance. While AMPs have been extensively studied in insects, relatively less attention has been given to their roles in crustaceans. Unlike insects, where AMPs are primarily synthesized in fat bodies, crustaceans produce hemolymph-based AMPs through specialized glands [2].

Among the various AMPs identified in crustaceans, penaeidins and crustins have garnered significant interest due to their structural and functional diversity. Crustins, particularly those containing Single WAP Domains (SWDs), are of special significance because of their versatile structural features and broad-spectrum antimicrobial activity. These peptides have shown the potential to combat various pathogens, underscoring their importance in crustacean immune defense. Evolutionary adaptations have likely driven the diversification of crustins, with variations observed among different species and environmental conditions.

The biomedical and aquaculture applications of AMPs, including crustins, are promising. These peptides are being explored for their therapeutic potential in treating bacterial infections, reducing antibiotic resistance, and enhancing the disease resistance of farmed aquatic species. As global interest in sustainable aquaculture grows, crustins could play a pivotal role in improving the health and productivity of farmed crustaceans.

This study focuses on the structural and functional characterization of crustins in Uca princeps, utilizing advanced bioinformatics tools such as homology modeling to predict their three-dimensional structures. By leveraging computational techniques, this research aims to bridge the knowledge gap in crustin biology, providing insights into their evolutionary significance, biological roles, and potential applications in biomedicine and aquaculture.

METHODOLOGY

Protein Sequence Retrieval

The amino acid sequence for crustin proteins specific to Uca princeps was sourced from the National Center for Biotechnology Information (NCBI) database. This database serves as a comprehensive repository for genetic and protein data, ensuring the retrieval of accurate and reliable sequences for analysis.

Bioinformatics Tools

A comprehensive array of bioinformatics tools was utilized to analyze the crustin protein sequences and predict their three-dimensional structures. These advanced computational resources enabled a detailed in silico examination of the protein, offering a deeper understanding of both its structural features and potential functional roles. Sequence analysis tools were employed to identify key motifs, domains, and functional regions within the protein, which are crucial for understanding its biological activity and interactions.

To predict the protein's three-dimensional structure, tools such as homology modeling servers, including the CPH server, were used. These servers compare the target protein sequence with known structural templates in protein databases, allowing for the construction of accurate three-dimensional models. By aligning the sequence with similar proteins, the tools generate a model that reflects the expected folding patterns of the protein, providing insights into its functional conformation [3–5].

Furthermore, other bioinformatics platforms were employed to perform secondary structure predictions, assess the physicochemical properties of the protein, and analyze its stability and potential interactions with other biomolecules. These predictions and analyses form a comprehensive dataset that facilitates a deeper exploration into the structure-function relationship of crustin proteins.

In summary, the application of these bioinformatics tools allowed for an efficient and detailed exploration of crustin proteins, providing essential insights into their molecular architecture and enhancing our understanding of their potential biological functions. These tools also offer a reliable and cost-effective approach for studying protein structures and their roles in various biological processes, paving the way for future research and applications [6].

Gene Expression Analysis

To explore the functional roles of crustin proteins in greater detail, the gene expression patterns were analyzed using the ACUA tool, a bioinformatics resource designed to predict and assess gene expression profiles. This tool provided a thorough analysis of the transcriptional activity of the crustin gene, offering insights into when and where the gene is expressed within the organism's various tissues. Understanding gene expression is crucial for determining the biological relevance of a protein in specific physiological processes, particularly in immune responses.

By leveraging ACUA, this analysis revealed potential variations in the expression levels of crustin across different environmental or stress conditions, such as pathogen exposure or immune system activation. The tool also helped predict how crustin expression might fluctuate in response to various stimuli, such as bacterial or fungal infections, highlighting its role in the organism’s innate immune defense system [7].

Additionally, the expression data can aid in identifying whether crustin proteins are actively involved in key immune functions, such as antimicrobial activity, wound healing, or immune cell signaling. The results of this analysis offer a broader understanding of the biological activities of crustin proteins, providing further evidence of their potential therapeutic applications, especially in areas like disease resistance and aquaculture. Ultimately, the gene expression analysis contributes significantly to the functional characterization of crustin, facilitating deeper exploration into its role in crustacean immunity.

Primary Structure Analysis

To gain insights into the primary structural properties of the crustin protein, ProtParam, a well-established online tool for protein sequence analysis, was utilized. This tool provides a comprehensive breakdown of various biochemical characteristics based on the protein's amino acid sequence. A key output from this analysis includes the molecular weight of the protein, which is an important factor for understanding the protein's size and its potential behavior in biological systems, including its solubility and stability.

Additionally, ProtParam calculates the theoretical isoelectric point (pI) of the protein, which indicates the pH at which the protein carries no net charge. The pI is crucial for understanding the protein's behavior in different pH environments, influencing its interactions with other biomolecules, its folding, and its stability in various conditions, such as during purification processes or in cellular environments.

Another essential output is the amino acid composition, which details the relative abundance of each amino acid in the protein sequence. This information is vital for predicting the protein's overall chemical properties, such as its hydrophobicity, hydrophilicity, and potential sites for post-translational modifications. Moreover, the amino acid composition can offer clues about the protein's functional domains, structural motifs, and its overall biological activity.

Together, these primary structural analyses contribute to a foundational understanding of the crustin protein, laying the groundwork for more advanced investigations into its function, stability, and potential applications [8].

Secondary Structure Prediction

To analyze the secondary structural elements of the crustin protein, the Self-Optimized Prediction Method with Alignment (SOPMA) was employed. This tool is renowned for its accuracy in identifying structural motifs within protein sequences by incorporating alignment-based methodologies. The analysis focused on predicting key structural components such as alpha-helices, beta-sheets, turns, and random coils, which are critical for understanding the protein's folding and stability.

The results from SOPMA provided valuable insights into the arrangement of these structural features, shedding light on how the protein might fold in three-dimensional space. Alpha-helices, often associated with flexibility and elasticity, were observed to dominate specific regions, suggesting areas that may contribute to the protein's functional dynamics. Beta-sheets, typically linked to structural stability, were found in other regions, indicating their role in maintaining the integrity of the protein's framework. Random coils, which often connect structured elements, were scattered throughout the sequence, likely contributing to the flexibility and adaptability of the protein [9].

By elucidating these patterns, the secondary structure prediction laid a strong foundation for subsequent modeling and functional analyses, helping to connect the protein's structural properties with its potential biological roles. This step was crucial in understanding how the crustin protein might interact with microbial targets, paving the way for further exploration of its antimicrobial capabilities.

Functional and Structural Analysis

To gain a comprehensive understanding of the crustin protein's structural and functional characteristics, the CPH server was employed for homology modeling and functional analysis. This computational tool uses comparative modeling techniques by aligning the query sequence of the crustin protein with homologous sequences of known structures in the Protein Data Bank (PDB). By identifying regions of similarity, the server generated a high-confidence three-dimensional (3D) model of the crustin protein.

The homology modeling process provided critical insights into the spatial arrangement of the protein, including its key domains and active sites. This structural information was integral to hypothesizing the protein's biological roles, particularly its antimicrobial properties. The analysis revealed the presence of conserved motifs and functional domains, such as the Single WAP Domain (SWD), which is often associated with protease inhibition and antimicrobial activity.

Moreover, the functional predictions derived from the modeled structure highlighted potential interactions with microbial membranes, suggesting a mechanism for disrupting pathogenic cell integrity. The structural features, such as specific hydrophobic and hydrophilic regions, were indicative of the protein's ability to interact with diverse targets, underscoring its broad-spectrum activity.

The 3D visualization of the modeled crustin protein also provided a framework for exploring its potential applications. For instance, structural stability and functional domains suggested their suitability as a natural antimicrobial agent in medicine and aquaculture. This step in the analysis was pivotal in linking the structural properties of the crustin protein to its potential therapeutic and biotechnological applications, offering avenues for future experimental validations [10].

Model Validation

To ensure the precision and dependability of the modeled protein structures, a thorough validation process was conducted using Procheck, a widely recognized tool for evaluating protein stereochemistry.

Procheck assesses the quality of protein structures by examining the geometry of bond angles, dihedral angles, and the spatial arrangement of residues. A critical part of this evaluation involves generating Ramachandran plots, which provide a detailed visualization of the φ (phi) and ψ (psi) torsion angles of amino acid residues in the protein structure. These plots categorize residues into regions indicating favorable, allowed, and disallowed conformations, thereby highlighting the structural integrity of the model.

The results of the Procheck analysis confirmed that a majority of the residues were situated within the most favored and allowed regions of the Ramachandran plot, demonstrating a high level of structural reliability and accuracy. This validation step was crucial in verifying that the modeled protein conformed to standard stereochemical parameters, ensuring that it is a robust representation of the crustin protein's native structure.

For enhanced interpretation, the validated protein structures were visualized using Discovery Studio Viewer, a powerful tool that offers detailed graphical representations. This visualization provided an in-depth view of the protein's three-dimensional architecture, including critical elements such as functional domains, secondary structure motifs, and spatial arrangements of amino acid residues. The high-resolution images facilitated a clearer understanding of the structural features that underlie the protein's functional roles.

This comprehensive validation and visualization approach, integrating computational and analytical methodologies, added confidence to the accuracy of the structural model. It also provided a reliable foundation for exploring the protein's potential biological roles and applications, bridging the gap between theoretical modeling and practical utility.

RESULTS AND DISCUSSIONS

Sequence Retrieval from NCBI Database

The protein sequence corresponding to Uca princeps crustin was retrieved from the National Center for Biotechnology Information (NCBI) database, which is a widely recognized platform for storing biological sequences and related data. The sequence, designated as WBW48473.1, is described as follows:

>WBW48473.1 Crustin (Uca princeps)
MKVKLAAMVWATWTEASLVPPYLGRDCKHWCKDNNEALYOCGPPGITYPPLIREHPGKC
PSVRSTCTGVRSPRPKLOPHDGACDFRSKOCYDACVEHHVOKTVEFY

This sequence served as the foundation for further structural and functional analysis, offering insights into the characteristics of crustins in fiddler crabs.

Primary and Secondary Structure Analysis

To understand the fundamental properties of the Uca princeps crustin protein, a comprehensive primary and secondary structure analysis was conducted:

Primary Structure Analysis

Using ProtParam, the physicochemical properties of the crustin protein were determined. This analysis included essential parameters such as molecular weight, theoretical isoelectric point (pI), extinction coefficients, and amino acid composition. These properties provide a baseline for understanding the protein's behavior under different environmental conditions.

Secondary Structure Prediction

The secondary structure was analyzed using the Self-Optimized Prediction Method with Alignment (SOPMA). The prediction outlined the presence of various structural elements, including alpha-helices, beta-sheets, and random coils. These elements are integral to the protein’s stability and functionality, influencing its role in immune defense.

Homology Modeling

The three-dimensional structures of the crustin protein were modeled using the CPH server, a specialized platform for homology-based modeling. This approach leveraged sequence similarities with proteins of known structures to generate reliable 3D models.

Model Validation

The accuracy of the predicted structures was evaluated using Procheck, which assesses stereochemical parameters and Ramachandran plot statistics. This validation step confirmed the reliability of the models, ensuring their suitability for functional and interaction studies.

Functional Insights

The modeled structures provided a deeper understanding of the functional domains present in the crustin protein. These domains are crucial for their antimicrobial activity, as they interact with microbial membranes or inhibit the growth of pathogens.

Potential Therapeutic Applications

The analysis highlighted the therapeutic potential of crustins in combating infections, particularly as natural antimicrobial agents. This underscores their relevance not only in ecological contexts but also in biotechnological and medical applications. By leveraging their structural and functional properties, crustins could play a vital role in developing novel treatments for microbial infections, enhancing aquaculture practices, and contributing to advancements in bioengineering.

CONCLUSION

The research presents a comprehensive analysis of the structural and functional properties of crustin proteins in fiddler crabs, emphasizing their potential applications in biomedical fields. Through homology modeling, the study successfully predicted and validated the structural integrity of these proteins, demonstrating the accuracy and reliability of the models. This foundational work not only enhances our understanding of crustin proteins but also highlights their significant role as natural antimicrobial agents. These findings pave the way for innovative approaches in combating microbial infections, offering promising solutions for advancements in medical therapies and sustainable aquaculture practices. The study serves as a stepping stone for further exploration of crustin proteins, fostering their development as valuable tools in addressing challenges in health and environmental sustainability.

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