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Research article

Characterization of the ligand binding of PGRP-L in half-smooth tongue sole (*Cynoglossus semilaevis*) by molecular dynamics and free energy calculation

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ABSTRACT

Background: Peptidoglycan (PGN) recognition proteins (PGRPs) are important pattern recognition receptors of the host innate immune system that are involved in the immune defense against bacterial pathogens. PGRPs have been characterized in several fish species. The PGN-binding ability is important for the function of PGRPs. However, the PGRP-PGN interaction mechanism in fish remains unclear. In the present study, the 3-D model of a long PGRP of half-smooth tongue sole (*Cynoglossus semilaevis*) (csPGRP-L), a marine teleost with great economic value, was constructed through the comparative modeling method, and the key amino acids involved in the interaction with Lys-type PGNs and Dap-type PGNs were analyzed by molecular dynamics and molecular docking methods.

Results: csPGRP-L possessed a typical PGRP structure, consisting of five β-sheets and four α-helices. Molecular docking showed that the van der Waals forces had a slightly larger contribution than Coulombic interaction in the csPGRP-L-PGN complex. Moreover, the binding energies of csPGRP-L-PGNs computed by MM-PBSA method revealed that csPGRP-L might selectively bind both types of MTP-PGNs and MPP-PGNs. In addition, the binding energy of each residue of csPGRP-L was also calculated, revealing that the residues involved in the interaction with Lys-type PGNs were different from that with Dap-type PGNs.

Conclusions: The 3-D structure of csPGRP-L possessed typical PGRP structure and might selectively bind both types of MTP- and MPP-PGNs, which provided useful insights to understanding the functions of fish PGRPs.

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1. Introduction

Pattern recognition receptors (PRRs) are important molecules of innate immunity that can specifically recognize conserved molecular patterns present in pathogens but absent in the host [1]. To data, a number of PRRs have been identified in teleosts, including Toll-like receptors [2], retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) [3], NOD-like receptors (NLRs) [4], C-type lectin [5], and peptidoglycan recognition proteins (PGRPs) [6].

PGRPs were first purified from the silkworm *Bombyx mori* hemolymph according to their high affinity to peptidoglycan (PGN), the essential cell wall component of almost all bacteria [7]. Then, PGRPs were identified from several invertebrates and vertebrates. Depending on the length of their amino acids, PGRPs were divided into three types: short PGRPs (PGRP-S), long PGRPs (PGRP-L), and intermediate PGRPs (PGRP-I). The short and long PGRPs are present in all species, while intermediate PGRPs were only reported in mammals [6].

All the PGRPs possess at least one conserved PGRP domain at their N-terminals, which is approximately 160 amino acids in length. Structurally, PGRPs are similar to type 2 bacteriophage amidases, containing three peripheral α-helices and several central β-sheet strands [6]. The front face of the PGRPs form a PGN-binding groove, and many amino acid residues that are important for the functioning of PGRPs are found in this groove [8]. However, different PGRPs exhibit different PGN-binding ability toward Lys-PGN and Dap-PGN. For example, *Drosophila* PGRP-SA could bind Lys-PGN [9], while PGRP-LC and PGRP-LE bind Dap-PGN [10]. Similar results were also found in mammalian PGRPs. Human PGLYRP1 could bind Lys-PGN and Dap-PGN [11], while PGLYRP3 only bind Lys-PGN [12]. Structural analysis of PGRPs provide solid basis for fully understanding the specific mechanism for PGRPs binding different PGNs.

Fish are lower vertebrates inhabiting the aquatic environment and serving as an essential link to early vertebrate evolution. It had been found that fish might possess a more complex immune system than

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previously believed [13,14]. To date, PGRPs have been identified in several fish species, such as zebrafish [15], grass carp [16], and rainbow trout [17]. Fish PGRPs have PGN-binding ability, amidase activity, and direct bactericidal activity [15,16,17]. However, the molecular basis for the interaction between fish PGRPs and PGNs remain unclear.

Homology modeling method is a convenient method for constructing an atomic resolution model of the protein from its amino acid sequence and an experimental 3-D structure of a related homologous protein. Using this method, the structures of many important immune molecules, e.g., interleukin (IL)-22 [18] and nucleotide binding and oligomerization domain (NOD) 2 [19], had been constructed, and the interactions with their ligands were also elucidated.

In the present study, the 3-D structure of a long PGRP from half-smooth tongue sole (*Cynoglossus semilaevis*) (csPGRP-L), a marine teleost with great economic value in China and other Asian countries [20], was constructed using the comparative modeling method. The molecular interaction between csPGRP-L and PGNs were studied using molecular docking, molecular dynamics (MD) simulations, and molecular mechanics/Poisson Boltzmann surface area (MM/PBSA) methods. This study elucidates the structural and dynamics properties of csPGRP-L and the molecular basis for csPGRP-L-PGN complex, giving first insights into the PGRP-PGN interaction mechanism in teleosts.

2. Materials and methods

2.1. Homology modeling and MD analysis of csPGRP-L

The crystal structures of human peptidoglycan recognition protein PGRP-S (PDB code: 1YCK) was selected as the template protein to build the structure of csPGRP-L. Homology modeling was performed using the Prime module of Schrödinger software [21]. Ramachandran plot was used to test the reliability of the structure.

The MD simulation system of csPGRP-L was built and run using GROMACS package 5.1 using AMBER99SB force field [22]. The amino groups were fully protonated (Lys, Arg, and N-terminal), and the carboxylic groups were deprotonated (Asp, Glu, and C-terminal). The protein was placed in a cubic box whose surface to the closest atom of the solute was set to 1.2 nm. Subsequently, TIP3P water molecules were filled in the box, and the system was neutralized with 0.10 M NaCl. The obtained system was first energy minimized using the steepest descent algorithm to remove steric clash. Then, 200 ps NVT (constant temperature, constant volume ensemble) and 500 ps NPT (constant temperature, constant pressure ensemble) MD simulations were carried out with position restrictions on protein successively. Q6 Finally, the production MD was run for 30 ns at 300 K using the V-rescale method. The pressure was kept at 1 atm using a Parrinello-Rahman barostat. Long-range electrostatic interaction was considered using the particle mesh Ewald method. Trajectories were saved every 20 ps. The final structure was extracted and used for further docking calculations.

2.2. Molecular docking of PGN ligands with csPGRP-L

The minimized structure of csPGRP-L from MD simulation was adopted as receptor. The binding site was identified using SiteMap module [23], and the receptor grid was generated using the obtained binding site with a box size of 20 Å. The ligand structures of muramyl tripeptide (MTP), muramyl tetrapeptide (MTrP), and muramyl pentapeptide (MPP) were taken from PDB databank (PDB ID: 1TWQ, 4KNL, and 2APH, respectively). The lysine residues at the third position were replaced with Dap using Maestro build tools. The structures of ligands were prepared using the LigPrep module [24]. The possible ionization state at pH 7.0 ± 2.0 was determined using the Epik method. The OPLS-2005 forcefield was used to produce the low-energy conformer. Molecular docking calculations were carried out using the Glide module of Schrödinger software at standard precision [25].

2.3. Binding free energy calculation

Six csPGRP-L-PGN complexes obtained by molecular docking were used as the initial structures for MD simulations. The topology parameters of ligands were derived using the RESP (Restrained ElectroStatic Potential) method with General Amber Force Field. The calculation steps and parameter setup were the same as that of csPGRP-L calculation. Production of MD simulation lasting for 20 ns were performed for each complex. The binding free energies between PGN fragments and csPGRP-L were calculated using the MM/PBSA method. MM-PBSA calculations were performed on the last 5-ns trajectories by using g_mmpbsa tool embedded in GROMACS software [26]. The selected nonpolar solvation model is based on the solvent-accessible surface area (SASA) with probe radius 1.4 Å.

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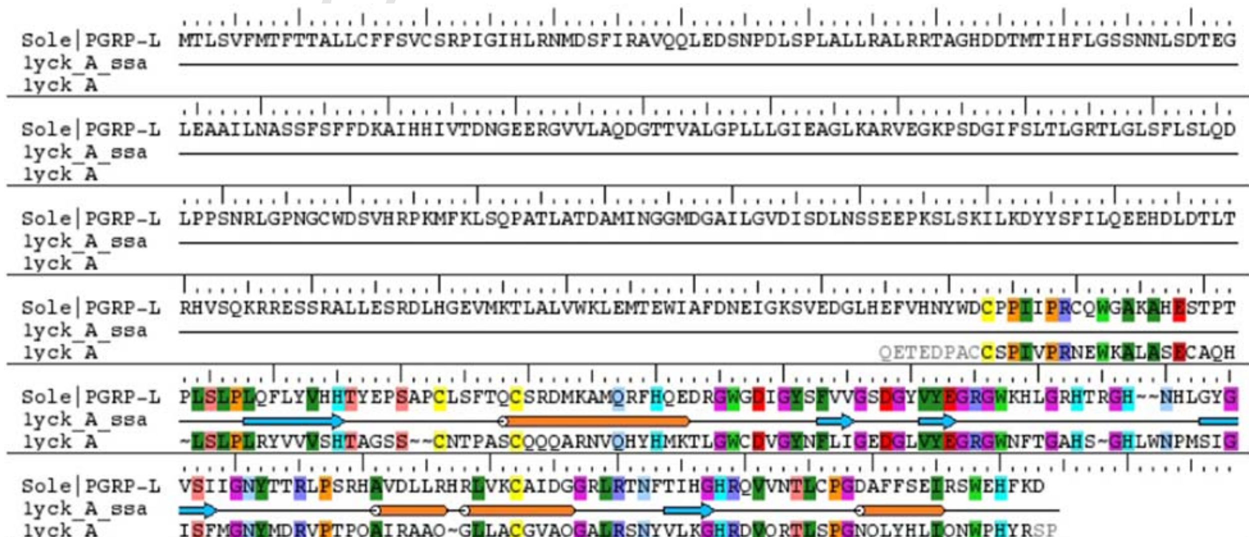

Fig. 1. Sequence alignment of LPGPR with the template PDB 1YCK.

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