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Purification, characterization and cDNA cloning of a novel lipopolysaccharide-binding lectin from the shrimp *Penaeus monodon*☆

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Abstract

In invertebrates, C-type lectin plays an important role in innate immunity by mediating the recognition of pathogens to host cells and clearing microinvaders. A few C-type lectins have been identified from shrimps, but none of their gene or protein sequences is known to date. In this paper, a C-type lectin (named PmLec) specific for bacterial lipopolysaccharide was purified from the serum of the shrimp *Penaeus monodon*. The binding of PmLec to lipopolysaccharide was mainly mediated through the O-antigen. PmLec had a strong hemagglutinating and bacterial-agglutinating activity as well as an opsonic effect that enhances hemocyte phagocytosis. The PmLec cDNA sequence was obtained from the cDNA library of *P. monodon* by polymerase chain reaction with the degenerated primer designed according to the amino-terminal residue sequence of purified PmLec. A 546-bp open reading frame was found to encode a putative protein comprising 182 amino acids and containing a preceding signal peptide of 17 amino acids. A C-type lectin domain existed in PmLec, but no glycosylation site was found. The recombinant PmLec protein expressed in *Escherichia coli* also showed the same agglutinating activity and opsonic effect as that of the native protein. This is the first report of a lectin cDNA from the shrimp. PmLec functions as a pattern-recognition protein and an opsonin in the shrimp, and it provides a clue to elucidate the role of lectin in the innate immunity of aquatic invertebrates at the molecular level.

Keywords: Lectin; Shrimp; Lipopolysaccharide; cDNA; Opsonin; Innate immunity

1. Introduction

Innate immunity not only in vertebrates but also in invertebrates is now attracting a significant amount of attention. Unlike vertebrates, invertebrates are believed to lack adaptive immunity and to rely completely on their innate immune system including a set of humoral and cellular immune reactions [1]. Recognition of nonself materials in the innate immune system is mediated by a group of proteins named pattern recognition proteins (PRPs), which recognize and bind to different molecules on the surface of invading microorganisms. Accordingly, these surface molecules are designated as pathogen-associated molecular patterns (PAMPs). Binding of PRPs to PAMPs triggers a series of immune responses, leading to the activation of the host-defense system. PRPs include lectins, lipopolysaccharide (LPS)-binding proteins,

Abbreviations LPS, lipopolysaccharide; TB, Tris buffer; TBS, Tris-buffered saline; TBS-Ca, Tris-buffered saline with calcium; Nano-ESI-MS/MS, nanoelectrospray ionization tandem mass spectrometry; ORF, open reading frame; NTA, nitrilotriacetic acid; CTLD, C-type lectin domain.

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peptidoglycan-binding proteins, and β-1,3-glucan-binding proteins, and so on [2,3].

Lectins are proteins that have the ability to bind to specific carbohydrates and they are present in almost all living organisms. Lectins have been known as playing a central role in nonself recognition and clearance of invaders in invertebrate immunity [4,5]. Some lectins from invertebrates were reported to be involved in various biological responses, for instance promotion of phagocytosis [6–8], antibacterial activity [9–11], activation of the proPO system [12–14] and nodule formation [15].

From shrimps, a few lectins have been purified and characterized [16–19], but no lectin genes have been identified to date. In this paper we report the purification, characterization, cDNA cloning, and recombinant expression of a novel LPS-binding lectin (PmLec) from the shrimp *Penaeus monodon*. The molecular information of PmLec might provide a clue for further study on the mechanism of effect of lectin on the innate immunity of aquatic invertebrates, which would also be helpful in controlling shrimp diseases.

2. Materials and methods

2.1. Shrimps

Live *P. monodon* were purchased from a supermarket in Xiamen, China. Hemolymph was taken from the ventral sinus with a 1-ml sterile syringe and centrifuged at 15,000*g* for 15 min at 4 °C to separate the clot from the serum.

2.2. Affinity chromatography

A Sepharose-4B (Amersham) column was equilibrated with Tris-buffered saline (TBS) (50 mM Tris–HCl, pH 7.5, 100 mM NaCl). Shrimp serum was loaded onto the column; then the column was washed with TBS and eluted with 0.1 M glycine, pH 3. The eluate was analyzed by 14% (w/v) SDS-PAGE.

2.3. Hemagglutination and bacterial agglutination assay

A 2% (v/v) suspension of a variety of animal erythrocytes was prepared in Tris-buffered saline with calcium (TBS-Ca) (50 mM Tris–HCl, pH 7.5, 100 mM NaCl, and 10 mM $CaCl_2$). The sample (25 μl) of 2-fold serial dilutions in the same buffer was mixed with 25 μl of the cell suspension in 96-well microtiter plates (Corning), and hemagglutination was observed after incubation for 1 h at 25 °C under a microscope (Olympus). To analyze whether the hemagglutination requires calcium, we added different concentrations of EGTA to the TBS-Ca. To analyze whether the hemagglutination can be recovered by calcium, we mixed 10 mM EGTA and 10 mM $CaCl_2$.

For a bacterial agglutination assay, tested bacteria ($A_{600}=1.6$) were resuspended in TBS-Ca at 2.5×10^9 cells/ml respectively, and agglutinating activities were assessed by using the same method described above for the erythrocyte hemagglutination assay. Bacterial species *Escherichia coli* DH5α, *Bacillus subtilis*, *Staphylococcus aureus*, and yeast *Candida albicans* were kind gifts from Dr. Dezan Ye (the Third Institute of Oceanography of SOA, China). *Micrococcus lysodeikticus*, *Pseudomonas fluorescens*, *Aeromonas hydrophila*, *Vibro alginolyticus*, and *Vibro parahaemolyticus* were kind gifts from Dr. Xuanxian Peng (Xiamen University, China).

2.4. Hemagglutination inhibition assay

To test carbohydrate specificity for PmLec, we premixed 12.5 μl of serial dilutions of various carbohydrates (Sigma) in TBS-Ca with 12.5 μl of PmLec solution (∼8 μg/ml) for 30 min at 25 °C before adding the rabbit erythrocytes suspension. An inhibitory effect was expressed as the minimum concentration each carbohydrate required for complete inhibition of the hemagglutinating activity of PmLec.

2.5. LPS-binding assay

Wells of a flat-bottomed, 96-well assay plate (Corning) were coated with LPS from *E. coli* O127:B8 (Sigma) by a method modified from Yu et al. [12]. Briefly, LPS was suspended at 40 mg/ml in water and sonicated 3 times for 15 s; 50 μl (2 mg) of LPS suspension was then added to each well. The plate was incubated at room temperature until the water evaporated completely, heated at 60 °C for 30 min, and then blocked with 200 μ/well of 1 mg/ml BSA in Tris buffer (TB) (50 mM Tris–HCl, pH 8.0, 50 mM NaCl) for 2 h at 37 °C. The plates were then rinsed four times with 200 μ1/well of TB. Different concentrations of PmLec diluted with TB containing 5 mM $CaCl_2$ and 0.1 mg/ml BSA were added at 50 μl/well, and binding was allowed to occur for 3 h at room temperature. The plates were rinsed four times with 200 μl/well of TB, and mouse anti-PmLec antiserum (diluted 1000-fold with TB containing 0.1 mg/ml BSA) was then added at 100 μl/well. After incubation for 2 h at 37 °C, the wells