Fish and Shellfish Immunology 77 (2018) 131–138

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

The effect of the lectin from *Cherax quadricarinatus* on its granular hemocytes

José Luis Sánchez-Salgado[a,b,*], Mohamed Alí Pereyra[a], Concepción Agundis[a], Oscar Vivanco-Rojas[a], Carlos Rosales[c], Cristina Pascual[d], Juan José Alpuche-Osorno[e], Edgar Zenteno[a,f]

[a] Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico City, Mexico
[b] Posgrado de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Mexico City, Mexico
[c] Departamento de Inmunología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
[d] Unidad Multidisciplinaria de Docencia e Investigación, Facultad de Ciencias, Universidad Nacional Autónoma de México, Sisal, Yucatán, Mexico
[e] CONACYT-Facultad de Medicina y Cirugía, Universidad Autónoma Benito Juárez de Oaxaca, Oaxaca, Mexico
[f] Centro de Investigaciones, Facultad de Medicina UNAM-Universidad Autónoma Benito Juárez de Oaxaca, Oaxaca, Mexico

ARTICLE INFO

Keywords:
Signaling pathways
Hemocyte activation
Cherax quadricarinatus
Immune system
Lectins
Membrane receptor

ABSTRACT

In crustaceans, lectins and hemocytes of the innate immune system provide the first line of defense. Although evidence points to the potential role of lectins in regulating hemocyte activity, the processes underlying the lectin activation have not been evaluated. In the present study, the receptor for CqL, a humoral lectin from *Cherax quadricarinatus* specific for galactose/sialic acid, was identified in a granular subset of hemocytes. The CqL receptor (CqLR) is a 490-kDa glycoprotein, composed of four identical 120-kDa subunits. As shown by immunohistochemistry, CqL at 7.5 μg/mL as optimal dose, after 2 min, induced, specifically on granular hemocytes, increased phosphorylation of serine (152%), threonine (192%), and tyrosine (242%) as compared with non-treated hemocytes; moreover, CqL induced increased generation of reactive oxygen species (ROS). Specific kinase inhibitors showed inhibition ($P < 0.001$) of ROS production induced by CqL. These results strongly suggest that CqL actively participated in the generation of ROS through kinases induced by a CqLR in a subset of granular hemocytes of the crayfish *C. quadricarinatus*. The results provide strong evidence that CqL activates, through specific granular hemocytes, receptors that modulate cellular functions in *C. quadricarinatus*.

1. Introduction

Numerous mechanisms are involved in the elimination of pathogens in crustaceans [1]. Humoral immunity includes the prophenoloxidase system, antimicrobial peptides, and lectins [2]. Cellular immunity in the hemolymph of crustaceans is accomplished mainly by three cellular populations of hemocytes: granular, semi-granular, and hyaline [3]. The main functions of hemocytes are encapsulation, phagocytosis, and oxidative burst [4,5]. These cells utilize pattern recognition receptors to identify pathogen-associated molecular patterns of groups of microorganisms [6,7].

Several studies have suggested that serum lectins participate in the regulation of immune mechanisms in crustaceans [8]. Previous reports have identified the presence of glycosylated homo-receptors for serum lectins in crustacean hemocytes and described their role in activation of the immunological process [9–12]. In *C. quadricarinatus* serum, A lectin, CqL, with specificity for sialic acid, galactose, and glycoproteins containing sialylated O-glycosidically linked glycans, such as bovine submaxillary mucin, has been purified by affinity chromatography [13]. Moreover, it has been showed that CqL recognizes its own hemocytes, and this interaction seems to be mediated by recognition of glycosylated sites of its own membrane receptor [13].

In crustaceans, serum lectins show the capacity to induce reactive oxygen species (ROS) production in hemocytes; however, it is not clear which signaling molecules are involved [9,10]. In mammals, several molecules have been pointed out that participate in activating the NADPH complex and produce ROS, the most relevant molecules are kinases such as phosphatidylinositol 3-kinase (PI3K), protein kinase C

* Corresponding author. Facultad de Medicina - UNAM, Ciudad de México, 04510, Mexico.
E-mail address: jlsanchezsalgado@gmail.com (J.L. Sánchez-Salgado).

https://doi.org/10.1016/j.fsi.2018.03.050
Received 15 December 2017; Received in revised form 15 March 2018; Accepted 28 March 2018
Available online 29 March 2018
1050-4648/

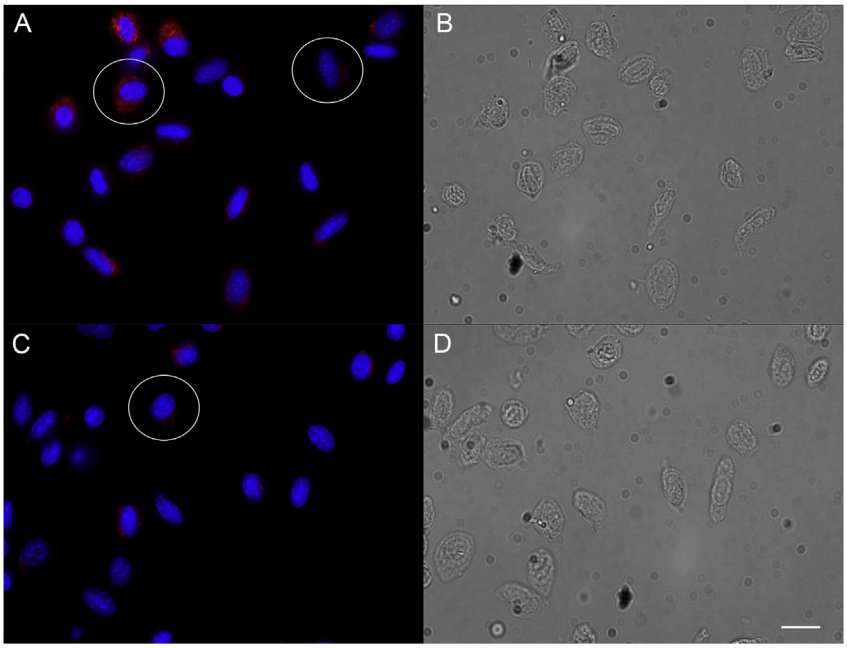

Fig. 1. *The CqL receptor in granular hemocytes. C. quadricarinatus* hemocytes were incubated with 5 μg/mL biotin-labeled CqL (B), or CqL previously incubated with 2.5 μM of BSM (D) and visualized with Alexa Fluor 594-labeled streptavidin. Hemocytes were observed in an inverted microscope under white light (B and D) or fluorescence (A and C). Granular hemocytes are marked with a white arrow. Nuclei were labeled with DAPI. Bar = 20 μm.

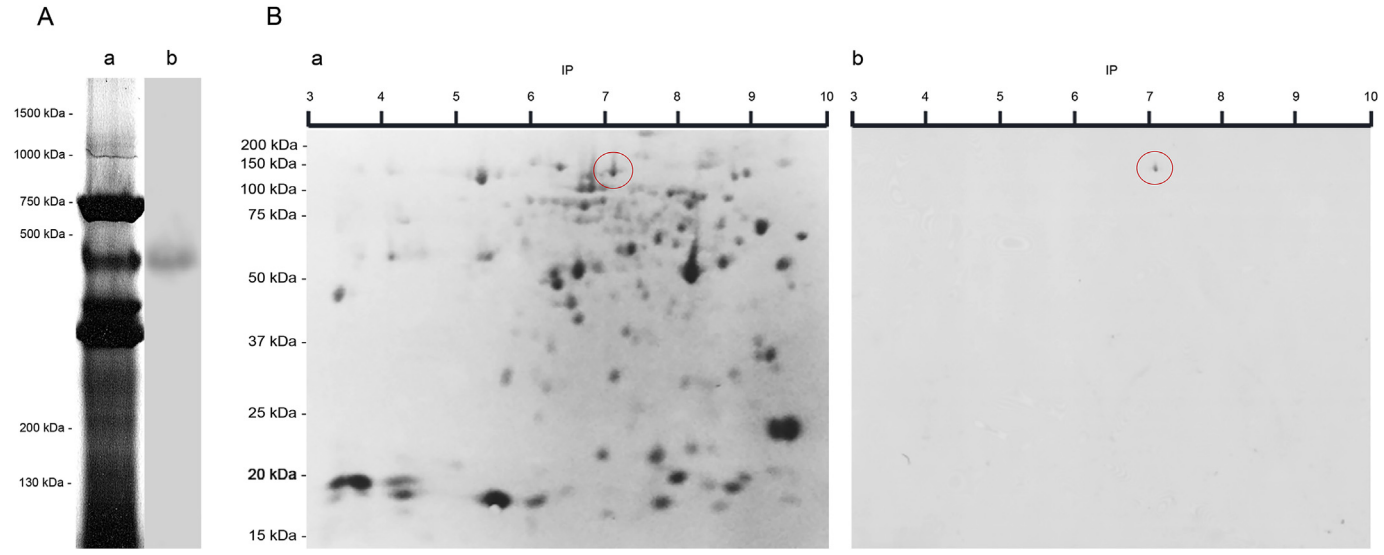

Fig. 2. *Partial characterization of the CqL receptor from hemocyte lysates*. A) Blue native electrophoresis of the CqLR. Hemocyte lysate proteins (150 μg) were stained with Coomassie Blue solution (Line a). Identification of native CqLR was revealed with CqL blotting as described in Materials and Methods (Line b). B) 2-D electrophoresis of the CqLR. Hemocyte lysate proteins (80 μg) were stained with Ponceau S solution (Fig. a). Identification of an isoform from CqLR revealed with CqL blotting (Fig. b).

Table 1
BLAST analysis of CqLR tryptic peptides.

Sequence	UniProtKB Accession number	Score	Identity
NSNDDAGVVVGR	Q86RQ4	70	83%
VVDGPGLTRPK	Q964D3	79	100%
DGTSPSGWTGSIK	Q964D3	94	100%
PFVLAEVNADVVR	Q6YNC7	96	100%

(PKC), tyrosine protein kinase (SYK), and mitogen-activated protein kinases (MAPK) [14]. In the present study, we characterized the CqL receptor and identified molecules that might participate in the signaling pathway and lead to the production of ROS in hemocytes of *C. quadricarinatus,* aimed at identifying better the role of serum lectins in crustaceans to regulate their immune functions.

2. Material and methods

2.1. Reagents

The following reagents were used: piceatannol, a spleen tyrosine kinase (Syk) inhibitor (Acros Organics, NY, USA); Gö6983, a protein kinase C (PKC) inhibitor (Calbiochem/EMD Millipore, MA, USA); LY294002, a phosphatidylinositol 3-kinase (PI3K) inhibitor (Calbiochem/EMD Millipore); and PD98059, a MEK (ERK kinase) inhibitor (Cell Signaling Technology, MA, USA). Acetylsalicylic acid (ASA) dexamethasone, sodium fluoride, and iodoacetamide (IAA) were purchased from Sigma Aldrich (MO, USA). Sodium azide was purchased from J.T. Baker (MA, USA). Albumin-free bovine immunoglobulin G IgG was purchased from Research Organics (OH, USA). All other chemicals, including SB203580 (a p38 mitogen-activated protein kinase (MAPK) inhibitor), zymosan A from *Saccharomyces cerevisiae*, NHS-