Fish and Shellfish Immunology 76 (2018) 58–67

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

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

Full length article

Ayu C-reactive protein/serum amyloid P agglutinates bacteria and inhibits complement-mediated opsonophagocytosis by monocytes/macrophages

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ARTICLE INFO

Keywords:
Ayu
Bacterial agglutination
Complement-mediated opsonophagocytosis
Monocyte/macrophage
Sequence analysis
Short-chain pentraxins

ABSTRACT

The short-chain pentraxins (PTXs), including C-reactive protein (CRP) and serum amyloid P (SAP), are soluble pattern recognition molecules (PRMs) that exhibit calcium-dependent binding to bacterial surface molecules. They opsonize pathogens or other particles by phagocytic clearance. However, the detailed functions of short-chain PTXs in teleosts remained unclear. In this study, we identified a short-chain PTX gene from ayu, *Plecoglossus altivelis*, and tentatively named as PaCRP/SAP. Sequence analysis revealed that PaCRP/SAP has typical characteristics of fish CRP/SAP and is mostly closely related to rainbow smelt (*Osmerus mordax*) SAP. PaCRP/SAP transcripts were detected in all tested tissues, with the highest level in the liver, and its expression significantly upregulated following *Vibrio anguillarum* infection. The active recombinant mature PaCRP/SAP (rPaCRP/SAPm) agglutinated Gram-negative bacteria (*Escherichia coli*, *V. anguillarum*, *Aeromonas hydrophila*, and *Vibrio parahaemolyticus*) and Gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*) in a calcium-dependent manner *in vitro*, and it correspondingly bound peptidoglycan and lipopolysaccharide in a dose-dependent manner. The binding of rPaCRP/SAPm to *E. coli* and *S. aureus* resulted in a clear inhibition of the deposition of ayu complement 3 (PaC3) on the bacteria. Furthermore, rPaCRP/SAPm decreased phagocytosis of rPaCRP/SAPm-bound *E. coli* and *S. aureus* cells by ayu monocytes/macrophages (MO/MΦ) in a complement-dependent way. However, rPaCRP/SAPm alone had no significant influence on phagocytosis. These results provided the first evidence that PaCRP/SAP might function in ayu immune responses via agglutinating bacteria and inhibiting complement-mediated opsonophagocytosis by MO/MΦ.

1. Introduction

The innate immune system recognizes microorganisms via a limited number of germline-encoded pattern-recognition receptors (PRRs). Based on cellular localization and function, PRRs are classified into two major groups: (1) cell-associated receptors, which are localized in different cellular compartments [1], and (2) soluble PRRs such as pentraxins (PTXs), which represent the functional ancestors of antibodies and are involved in pathogen opsonization, complement activation, and self versus modified self-discrimination [2]. PTXs, which belong to a family of animal lectins [3,4], are distinctively characterized by the presence of a highly conserved motif of known as PTX signature (HxCxS/TWxS) in their carboxy-terminal regions [5]. They are a key component of the humoral arm of innate immunity, processing functions in various pathophysiological conditions [6]. PTXs can be divided into two subclasses, the classical short-chain PTXs including C-reactive protein (CRP) and serum amyloid P (SAP), and the long-chain PTXs, which have an unrelated amino-terminal region coupled to a carboxy-terminal PTX-like domain [7].

In mammals, the short-chain PTXs are well characterized, both structurally and functionally. CRP and SAP are similarly composed of five identical subunits in a disc-like configuration and share extensive sequence homology, indicating that they evolved from a common ancestor [8]. They are involved in acute-phase response to trauma, injury or infection [9]. Furthermore, they can bind to various ligands in a characteristic calcium-dependent manner, such as DNA, chromatin, fibronectin, glycosaminoglycans, heparin, and the structures of microbial surfaces including lipopolysaccharide (LPS), peptidoglycan (PGN), and phosphorylcholine, etc [8,10–12]. Hence, CRP and SAP can also bind to a range of microbes and other particles such as apoptotic cells and dead cells to opsonize pathogens or cells by phagocytic clearance [9,13]. Previous reports have revealed that short-chain PTXs have affect the opsonophagocytosis of pathogens in two ways, by interacting directly with cell-surface Fcγ receptors (FcγR) or by affecting the classical complement pathway through several complement components (C1q, factor H, C4b-binding protein, C5b6 complex, etc.) [9,14–17].

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https://doi.org/10.1016/j.fsi.2018.02.038
Received 2 January 2018; Received in revised form 18 February 2018; Accepted 19 February 2018
Available online 23 February 2018

Mammalian CRP are reported to enhance opsonization, whereas the ultimate impacts of SAP are inconsistent, some show the induction of the opsonophagocytosis, and others show the inhibition [7,10,11,18–21].

In fish, some CRP and SAP were isolated from serum like plaice (*Pleuronectes platessa* L.), dogfish (*Mustelus cannis*), rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), and the striped catfish (*Pangasianodon hypophthalmus*) by using affinity chromatography and named based on their binding properties compared with mammalian homologues [22–29]. Recently, more cDNA sequences of CRP and SAP had been characterized in teleost, such as common carp (*Cyprinus carpio*), snapper (*Pagrus auratuse*), black rockfish (*Sebastes schlegelii*), rock bream (*Oplegnathus fasciatus*), half-smooth tongue sole (*Cynoglossus semilaevis*) and Atlantic salmon [30–37]. Fish encode several PTX-like molecules, but no clear separation of CRP and SAP by sequence homology and phylogenetic tree analysis is observed [37]. Hence, such short-chain PTXs in fish are tentatively named as CRP/SAP molecules [37].

Fish CRP/SAP are widely expressed in the tissues and cells of the innate immune system. Upon infection via a pathogen, their expressions increase dramatically and immediately [22,27,34–36,38]. Fish CRP/SAP can bind and agglutinate many bacteria, exhibiting calcium-dependent lectin activity [29,33,34]. However, detailed immunological functions of fish CRP/SAP were rarely reported. Only rainbow trout CRP and half-smooth tongue sole SAP are reported to be involved in the phagocytosis and host resistance to pathogen infections [28,36,39]. However, the exact mechanism of CRP/SAP in fish immune responses is still not clear.

Ayu (*Plecoglossus altivelis*) is an economically important fish in East Asia [40]. Pathogen infections have resulted in both production and animal welfare problems [41]. It is therefore important to study immune modulation of ayu against pathogens. In this study, we characterized a CRP/SAP gene from ayu (PaCRP/SAP), and analyzed its expression profile upon *Vibrio anguillarum* infection. In addition, its agglutination activity to bacteria and effects on opsonophagocytosis by monocytes/macrophages (MO/MΦ) were investigated.

2. Materials and methods

2.1. Fish rearing

Healthy ayu, weighing 40–50 g and without any pathological signs, were obtained from a fishery in Ninghai County, Ningbo City, China and healthy. Fish were kept in fresh-water tanks in a recirculating system at 20–22 °C for two-week acclimation prior to the experiments commencing. All experiments were performed according to the Experimental Animal Management Law of China and approved by the Animal Ethics Committee of Ningbo University.

2.2. Molecular cloning of PaCRP/SAP cDNA

The cDNA sequence of PaCRP/SAP gene was obtained from a previously sequenced ayu head kidney-derived MO/MΦ transcriptome [42] by using the BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and authenticated by further cloning and sequencing. The molecular weight (Mw) and isoelectric point (p*I*) was predicted using Compute p*I*/Mw tool (http://web.expasy.org/compute_pi/). N-glycosylation sites were predicted using NetNGlyc 1.0 server (http://www.cbs.dtu.dk/services/NetNGlyc/). Multiple alignments were analyzed using ClustalW (http://clustalw.ddbj.nig.ac.jp/). Phylogenetic and molecular evolutionary analyzes were conducted using MEGA version 7 [43]. Sequences used in this study are listed in Table 1.

Table 1
SAP and related CRP gene sequences used for analysis.

Accession number	Species		Gene
	Latin name	English name	
KP329195	*Plecoglossus altivelis*	ayu	CRP/SAP
NM_001124721	*Oncorhynchus mykiss*	rainbow trout	CRP/SAP-1a
FR904445	*O. mykiss*	rainbow trout	CRP/SAP-1b
FR904378	*O. mykiss*	rainbow trout	CRP/SAP-1c
FR904367	*O. mykiss*	rainbow trout	CRP/SAP-2
NM_001123671	*Salmo salar*	Atlantic salmon	CRP/SAP-1a
BT079947	*Esox lucius*	northern pike	SAP
BT074711	*Osmerus mordax*	rainbow smelt	SAP
JX914666	*Cynoglossus semilaevis*	half-smooth tongue sole	CRP
KT025856	*Cynoglossus semilaevis*	half-smooth tongue sole	SAP
GU441682	*Epinephelus coioides*	orange-spotted grouper	CRP
XM_006630971	*Lepisosteus oculatus*	spotted gar	SAP-1
XM_00396616	*Takifugu rubripes*	tiger puffer	SAP
BT082602	*Anoplopoma fimbria*	sablefish	SAP
AB665331	*Oplegnathus fasciatus*	rock bream	SAP-2
AB665328	*O. fasciatus*	rock bream	SAP-1
XM_005472765	*Oreochromis niloticus*	Nile tilapia	SAP
XP_004541469	*Neolamprologus brichardi*	fairy cichlid	SAP
XM_004541412	*Maylandia zebra*	zebra mbuna	SAP
CAAE01014679	*Tetraodon nigroviridis*	spotted green pufferfish	PTX
ENSDART00000105662	*Danio rerio*	zebrafish	CRP-1
ENSDART00000056381	*D. rerio*	zebrafish	CRP-2
AB028455	*Cyprinus carpio*	common carp	PTX
JQ010977	*C. carpio*	common carp	CRP-1
XM_019271265	*Larimichthys crocea*	large yellow croaker	CRP
XM_019271266	*L. crocea*	large yellow croaker	SAP
XM_020923671	*Boleophthalmus pectinirostris*	mudskipper	CRP
XM_020937340	*B. pectinirostris*	mudskipper	SAP
XM_004077855	*Oryzias latipes*	Japanese ricefish	CRP
FJ547474	*Ctenopharyngodon idella*	grass carp	PTX
NM_001639	*Homo sapiens*	human	SAP
M11725	*H. sapiens*	human	CRP
NM_011318	*Mus musculus*	mouse	SAP
X13588	*M. musculus*	mouse	CRP
NM_213887	*Sus scrofa*	pig	SAP
NM_213844	*S.scrofa*	pig	CRP
XM_002715288	*Oryctolagus cuniculus*	rabbit	SAP
L47237	*O. cuniculus*	rabbit	CRP
NM_001039564	*Gallus gallus*	chicken	CRP
XM_002198407	*Taeniopygia guttata*	zebra finch	SAP
XM_006115236	*Pelodiscus sinensis*	Chinese softshell turtle	CRP
XM_008122047	*Anolis carolinensis*	lizard	CRP
XM_003228401	*A. carolinensis*	lizard	SAP
NM_001008174	*Xenopus tropicalis*	tropical clawed frog	SAP
XM_002934081	*X. tropicalis*	tropical clawed frog	CRP-1
NM_001172215	*X. laevis*	African clawed frog	CRP-1
M14026	*Limulus polyphemus*	horseshoe crab	CRP-1.1
AY066022	*L. polyphemus*	horseshoe crab	SAP

2.3. Bacterial challenge

V. anguillarum was grown at 28 °C in nutrient broth with shaking, and harvested in the logarithmic phase of growth. Bacterial cells were washed, resuspended, and diluted to the appropriate concentration in sterile phosphate-buffered saline (PBS). Fish were intraperitoneally injected with 1.2×10^4 colony forming units (CFU)/fish *V. anguillarum*