Fish & Shellfish Immunology 39 (2014) 168–177

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Full length article

Sequence analysis and subcellular localization of crucian carp *Carassius auratus* viperin

Bing Wang, Yi-Bing Zhang*, Ting-Kai Liu, Jian-Fang Gui*

State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, University of Chinese Academy of Sciences, Wuhan 430072, China

ARTICLE INFO

Article history:
Received 19 February 2014
Received in revised form
17 April 2014
Accepted 30 April 2014
Available online 11 May 2014

Keywords:
Viperin
Carassius auratus
Cytoplasmic localization
Endoplasmic reticulum localization
Self-association

ABSTRACT

Human viperin is known as an interferon (IFN)-inducible antiviral protein and localizes to endoplasmic reticulum (ER) via its N-terminal amphipathic α-helix. Little is known about subcellular localization of fish viperin. Herein, we characterized subcellular localization of a fish viperin from crucian carp *Carassius auratus*. Crucian carp viperin is nearly identical to the other viperin proteins in sequence, with the exception of the first N-terminal 70 amino acids that are defined as N-terminal variable domain including an amphipathic α-helix. In addition to N-terminal variable domain, crucian carp viperin protein harbors a conserved middle radical SAM domain and a conserved C-terminal domain. Subcellular localization analyses indicate that crucian carp viperin is a cytoplasmic protein associated with ER. Sequence analyses reveal that amino acids 1–74 forms an amphipathic α-helix domain that drives ER-localization of crucian carp viperin. In addition, Coimmunoprecipitation assays show that crucian carp viperin proteins are able to self-associate. These results together indicate that similar to mammalian homologs, fish viperins likely play important roles in IFN response.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Virus infection elicits an innate immune response that is characterized of the activation of interferons (IFNs) and subsequent induction of hundreds of IFN-stimulated genes (ISGs) [1]. The products of ISGs limit the spread of viral infection, thus leading to establishment of host antiviral state. Among the hundreds of interferon-inducible proteins, there are only a few for which the detailed antiviral mechanism has been defined. Mx, dsRNA dependent protein kinase R (PKR), and 2′-5′-oligoadenylate synthetase/RNase L, are the earliest characterized ISGs for their antiviral mechanisms; however, triple gene deficiency in mice [2] and cell cultures [3] do not completely destroy host IFN antiviral response, suggesting that alternative pathways may also contribute to the IFN antiviral activity. In support of this notion, emerging evidence has revealed that many ISGs are implicated in IFN-mediated antiviral pathways. One of the currently characterized ISGs is viperin (virus inhibitory protein, endoplasmic reticulum-associated, IFN-inducible) that is an IFN-inducible antiviral protein and has a broad inhibitory ability to RNA and DNA viral replications [4–8].

Viperin is first identified as a virally-induced EST from primary human foreskin (HF) cells infected with human cytomegalovirus (HCMV), therefore also called cig5 (cytomegalovirus-induced gene no.5) [9]. Generally, viperin is not detectable in normal cells but it is significantly upregulated in response to viral infection, IFN and LPS treatment [4,9–12]. The produced human viperin protein resides exclusively in cytoplasm and is normally localized to endoplasmic reticulum (ER); however, HCMV infection causes the redistribution of viperin protein from ER-association first to the Golgi apparatus and then to cytoplasmic vacuoles [11,13]. Structurally, human viperin proteins are divided into three domains, a N-terminal variable domain that contains amphipathic α-helix, a middle domain characteristic of a conserved motif CxxxCxxC that exists in Radical SAM (Radical S-adenosylmethionine) enzymes, and a C-terminal conserved domain [14]. Structure and function analyses have revealed that the N-terminal amphipathic α-helix guides human viperin to localize to ER cytosolic face in normal cells, or to lipid droplets under IFN treatment or HCV infection [15,16]. Therefore, human viperin inhibits HCV replication by binding to lipid droplets

Abbreviations: IFN, interferon; viperin, virus inhibitory protein, endoplasmic reticulum-associated, IFN-inducible; NHD, N-terminal amphipathic alpha-helix domain; SAMD, radical S-adenosylmethionine domain; CCD, C-terminal conserved domain; ER, endoplasmic reticulum.

* Corresponding authors. Tel.: +86 27 68780663; fax: +86 27 68780123.
E-mail addresses: ybzhang@ihb.ac.cn (Y.-B. Zhang), jfgui@ihb.ac.cn (J.-F. Gui).

http://dx.doi.org/10.1016/j.fsi.2014.04.025
1050-4648/© 2014 Elsevier Ltd. All rights reserved.

to prevent viral assembly and/or budding [15,17]. Consistently, removal of N-terminal amphipathic helix domain of human viperin results in a homogenous cytoplasmic distribution when transfection of Huh-7 cells, which coincides with a loss of its ability to inhibit HCV replication [17]. In another paper, sequential deletion of the N-terminal region disrupts ER-association of human viperin in the transfected HEK-293T cells, resulting in its progressive accumulation in cytosol [16]. In addition, human viperin suppresses the replication of influenza virus by binding to farnesyl diposphate synthase and thus disrupting lipid rafts [13].

In fish, viperin is first identified as a viral hemorrhagic septicemia virus (VHSV)-induced gene in rainbow trout (*Oncorhynchus mykiss*) leukocytes [10]. Sequence analysis shows that all viperin proteins including fish counterparts are highly conserved except for about 70 N-terminal amino acids [14,18–20]. A recent report showed that tilapia (*Oreochromis niloticus*) viperin is significantly up-regulated by lipopolysaccharide (LPS) and poly(I:C), and seems to decrease bacterial numbers and alter the expression of immune-related genes when over-expression in fish muscle [21]. Fish viperin seems to be involved in antiviral response in mandarin fish *Siniperca chuatsi* by DNA vaccination against a rhabdovirus [22]. Interestingly, red drum *Sciaenops ocellatus* viperin displays differential expression patterns in response to intracellular and extracellular bacterial pathogens [20]. However, little is known about subcellular localization of fish viperins. In this study, we found that crucian carp viperin, previously identified from UV-inactivated grass carp reovirus (GCRV)-treated crucian carp *Carassius auratus* blastulae embryonic (CAB) cells [23,24], is a cytoplasmic protein and ER-association by transfection of GFP reporter vectors and immunocytochemistry assays. Further transfection of a panel of truncation mutants found that the N-terminal amino acids stretch 1–74 forms an amphipathic α-helix that is able to drive ER-localization of crucian carp viperin.

2. Materials and methods

2.1. Cells, virus and transfection

Crucian carp (*C. auratus*) blastulae embryonic cells (CAB) and grass carp (*Ctenopharyngodon idellus*) ovary cells (CO) were grown at 28 °C in medium 199 (Gibco) supplemented with 10% fetal calf serum (FCS), 100 units/ml penicillin and 0.1 mg/ml streptomycin [24]. HEK293T cells (ATCC Number: CRL-11268) were maintained at 37 °C, 5.0% CO_2 in DMEM (Gibco) supplemented with 10% FCS. Grass carp reovirus (GCRV), a dsRNA virus, was propagated in CAB cells according to our previous report [24]. Transfection experiments were performed as described previously [25]. In brief, CAB, CO or HEK293T cells seeded in six-well plates overnight were transfected with a mixture containing 1.6 μg plasmids and 4 μL lipofectamine 2000 (Invitrogen) in 0.5 mL FCS-free opti-MEM(Invitrogen) per well. At 6 h post transfection, the transfection mixture was replaced with 2 mL pre-warmed fresh medium followed by further experiments.

2.2. Gene cloning, and sequence analysis

The full length cDNA of crucian carp viperin genes (Genbank accession: AY303809) was cloned by RACE-PCR from CAB cells treated by UV-inactivated GCRV [24]. Multiple sequence alignments were performed by using ClustalW 1.8 program. Physico-chemical property of N-terminal amphipathic α-helix was analyzed using an online software HELIQUEST [26]. Helical wheel of the N-terminal amphipathic α-helix of amino acid residues 9–42 of viperin proteins were diagramed using a helical wheel projection program (http://rzlab.ucr.edu/scripts/wheel/wheel).

2.3. Plasmids

For analysis of self-association of crucian carp viperin proteins, the open reading frame (ORF) region of crucian carp viperin was cloned into *EcoR* I/*Xho* I sites of pcDNA3.1 (+) (Invitrogen, Carlsbad, CA). For subcellular localization by immunochemistry assays, the entire ORF or the N-terminal domain of viperin(NHD) was cloned into *Xho* I/*Kpn* I sites of pcDNA3.1 myc-His (-) vector (Invitrogen, Carlsbad, CA). For subcellular localization by transfection of GFP constructs, the fusion plasmid viperin-GFP was generated by cloning of the full-length ORF of crucian carp viperin into *EcoR* I/*Kpn* I sites of pEGFP-N3 vector (BD Biosciences Clontech). Similarly, the other fusion plasmids, NHD-GFP (amino acids 1–74), SAMD-GFP (75–238), CCD-GFP (238–359), N48-GFP(1–48), N97-GFP (1–97), N109-GFP(1–109), C245-GFP (115–359), C82-GFP (278–359), M54-GFP (67–120), M42-GFP (133–174), M229-GFP (49–277), were generated by cloning of different cDNA fragments corresponding to the indicated amino acid stretches into *EcoR* I/*Kpn* I sites of pEGFP-N3 vector. GFP-viperin, GFP-NHD (1–74) and GFP-CCD (238–359) in which the location of GFP is different from plasmids viperin-GFP, NHD-GFP and CCD-GFP, were generated by cloning of the indicated cDNA fragments into *EcoR* I/*Kpn* I sites of pEGFP-C1 vector (BD Biosciences Clontech). All the generated plasmids were verified by sequence analysis. The primers used are listed in Table 1.

2.4. Subcellular localization

HEK293T cells or CO cells were grown overnight to 80% confluence on microscopy cover-glasses in six-well plates, and then cotransfected with 0.8 μg of pDsRed2-ER (BD Biosciences Clontech) and 0.8 μg of GFP-constructs, or with 1.6 μg of GFP-constructs only. At 24 h post transfection, the transfected cells on the cover-glasses were fixed with 4% (v/v) paraformaldehyde for 20 min at room temperature, washed, incubated with 0.2% Triton X-100 for 30 min, finally stained with 4′,6-diamidino-2-phenylindole (DAPI) (100 ng/ml) for 10 min, followed by examination by confocal microscopy (Carl Zeiss MicroImaging) or fluorescence microscope (Nikon Eclipse 80i) according to previous reports [27,28]. The magnification of all images is ×400.

Table 1
Primers used for all of the studies.

Name	Sequence (5′ to 3′)	Usage
CaViperin -F	CGGGGTACCAGTGTCTACGCACCTTAACC	Plasmid construction
CaViperin -R	CCGCTCGAGATTGCCAGTCCTGTATGAC	
CaViperin-myc F	CCGCTCGAGAGTGTCTACGCACCTTAACC	
CaViperin-myc R	CGGGGTACCCCACTCCAGTTTCATCTCTTCCTTG	
GFP-CCD F	CCGGAATTCAAACCCAGTGCGCTGGAAGG	
GFP-CCD R	CGGGGTACCTCACCACTCCAGTTTCATCTCT	
Viperin-GFP F	CGGAATTCGTGTCTACGCACCTTAACCA	
Viperin-GFP R	GGGGTACCCCACTCCAGTTTCATCTCT	
NHD-GFP/myc R	CGGGGTACCGTTTACACTGCTTGGAGTGGTCAG	
SAMD-GFP F	CCGGAATTCATGTACCATTTTACCCGGCAGTGC	
SAMD-GFP R	CGGGGTACCTAGAGCAGTGATCTGCTCTGTC	
CCD-GFP F	CCGGAATTCATGAACCCAGTGCGCTGGAAGG	
N97-GFP R	CGGGGTACCGACGAACGAGGTCTTCGCAGTGTG	
N109-GFP R	CGGGGTACCAAGTCGTAAGCCTCGCTTTGCCTCTTC	
C245-GFP F	CCGCTCGAG CTGAAAGAAGCAGGAATG	
N48-GFP R	CGGGGTACCCCGTCCAAGAGGTGTTGGTTGC	
M54-GFP F	CCGGAATTCATG CTGACCACTCCAAGCAGT	
M54-GFP R	CGGGGTACCAAAGTTGATTTTTTCCATTCCTGC	
M42-GFP F	CCGGAATTCATGTTTCTGGGAGAGTTGGTGCGGTAC	
M42-GFP R	CGGGGTACCCAGAATGTCCA AGTAGTCACCG	
C82-GFP F	CCGGAATTC ATG CTGGACCGCCACCAGAGCGTCG	
M229-GFP F	CCGGAATTCATGAGTCGTCCGAAGACTCGCAC	
M229-GFP R	CGGGGTACCGAAGTCTTGGAATTGCTGATCGC	

Download English Version:

https://daneshyari.com/en/article/2431575

Download Persian Version:

https://daneshyari.com/article/2431575

Daneshyari.com