Cloning and characterization of hypusine-containing protein eIF5A from the olive flounder *Paralichthys olivaceus*

Hee Jeong Kong a,⁎, Gyeong-Eun Hong a, Woo-Jin Kim a, Young-Ok Kim a, Bo-Hye Nam a, Chang Hoon Lee b, Jeong Wan Do c, Jeong-Ho Lee d, Sang-Jun Lee e, Kyung-Kil Kim a

a Biotechnology Research Institute, National Fisheries Research and Development Institute, 408-1 Sirang-ri, Gijang-up, Gijang-gun, Busan 619-705, Republic of Korea
b Research Group for Marine and Silver Biotechnology, Pusan National University, 30 Jangjeon-dong, Geumjeong-gu, Busan 609-735, Republic of Korea
c Pathology Division, National Fisheries Research and Development Institute, 408-1 Sirang-ri, Gijang-up, Gijang-gun, Busan 619-705, Republic of Korea
d Genetics and Breeding Research Center, National Fisheries Research and Development Institute, 201 Dapo-ri, Namhu-myon, Geoje, Gyeongsangnam 656-842, Republic of Korea

A R T I C L E   I N F O
Article history:
Received 10 February 2009
Received in revised form 26 March 2009
Accepted 26 March 2009
Available online 1 April 2009

Keywords:
Eukaryotic translation initiation factor 5A
Hypusine-containing protein
Olive flounder
Paralichthys olivaceus
Viral hemorrhagic septicemia virus
GC-7

A B S T R A C T
Eukaryotic translation initiation factor 5A (eIF5A) is the only protein in eukaryotic cells that contains the unusual amino acid hypusine (N(epsilon)-(4-amino-2(R)-hydroxybutyl)-lysine). We isolated a 1385-bp eIF5A cDNA containing an open reading frame (ORF) of 468 bp, which encodes a protein of 155 amino acids with a conserved hypusine modification site, from the olive flounder Paralichthys olivaceus. Pairwise alignments revealed that flounder eIF5A had a high sequence identity with those of other known species including mammals. Real-time RT-PCR analysis showed the expression of eIF5A mRNA was constitutively detected in various tissues of healthy flounder. In HINAE cells or flounder kidney infected with the viral hemorrhagic septicemia virus (VHSV), the expression of eIF5A mRNA was slightly increased before cells showed cytopathic effects and then decreased when cells showed cytopathic effects. Treatment of N-guanyl-1,7-diaminoheptane (GC-7), a potent inhibitor of eIF5A hypusination, inhibited the expression of VHSV G protein in a dose-dependent manner suggesting a potential role for eIF5A and its hypusination in viral protein expression.

© 2009 Elsevier Inc. All rights reserved.

1. Introduction

Eukaryotic translation initiation factor 5A (eIF5A) is highly conserved, from yeast to mammalian cells, and is the only cellular protein that contains the unusual amino acid hypusine (N(epsilon)-(4-amino-2(R)-hydroxybutyl)-lysine). The hypusine residue is produced by the post-translational modification of a specific lysine (K*), which is embedded in a 12-amino acid hypusine core region (5-T-S-K-T-G-K*-H-G-H-A-K), in a two-step process involving deoxyhypusine synthase (DHS; EC 2.5.1.46) and deoxyhypusine hydroxylase (DOHH; EC 1.14.99.29) (Murphey and Gerner, 1987; Park et al., 1982; Park, 2006), soon after the translation of eIF5A mRNA. eIF5A also undergoes a post-translational modification catalyzed by transglutaminase (Beninati et al., 1995). In addition, it is a novel substrate of the Sir2-related deacetylase Hst2 in yeast, suggesting a possible acetylation of eIF5A (Shirai et al., 2008).

eIF5A was originally identified as a translational initiation factor according to its ability stimulating the formation of methionyl-puromycin, a dipeptide analog, *in vitro* (Kemper et al., 1976). eIF5A is essential for sustained proliferation and has been found in all eukaryotes examined as well as in archaeabacteria, but not in eu-bacteria (Chen and Liu, 1997; Park et al., 1993). Analysis of a novel eIF5A complex reveals that eIF5A is a regulator of p53 that may define a new pathway for p53-dependent apoptosis through the functional interaction with syntenin (Li et al., 2004). eIF5A is involved in the degradation of specific short-lived cellular mRNA in yeast (Valentini et al., 2002; Zuk and Jacobson, 1998). In addition, eIF5A functions as a cellular cofactor for HIV Rev and HTLV Rex transactivator proteins in transporting unspliced viral RNAs from the nucleus to the cytoplasm (Bevec and Hauber, 1997; Katabira et al., 1995).

Viral hemorrhagic septicemia virus (VHSV) belongs to the Novirhabdovirus genus of the Rhabdoviridae family, a group of bullet-shaped enveloped viruses that contain a single molecule of linear, negative-sense, single-stranded RNA (approximately 11.1 kb), coding for the nucleoprotein (N), the glycoprotein (G), and four other viral genes (3′-N-P-M-G-NV-L′-5′), which are expressed as individual transcripts (Schütze et al., 1999). VHSV is widely distributed among freshwater and marine fish and is an important pathogen because it is responsible for massive losses in the aquaculture of various farmed species including flounder (Hopper, 1999; Mortensen et al., 1999; Schlotfeldt and Ahne, 1988; Skall et al., 2003; Vestergård Jorgensen, 1982).

In the present study, we isolated and characterized eIF5A cDNA from the olive flounder Paralichthys olivaceus, and suggested that the
hypusine-containing eIF5A protein may be involved in the expression of VHSV G protein within the cell.

2. Materials and methods

2.1. Sequence analysis

We previously constructed cDNA library of the olive flounder Paralichthys olivaceus larval stage and carried out expressed sequence tag (EST) analysis (data not shown). A clone, FLDS-6-G2, was isolated using a plasmid miniprep kit (Qiagen) and was sequenced using T3 forward and T7 reverse primers (Promega) with an automatic sequencer, ABI3730xl (Applied Biosystems, Inc.). The cDNA sequence was annotated using BLASTX (http://www.ncbi.nlm.nih.gov/BLAST/) within GenBank.

2.2. Phylogenetic analysis

A multiple sequence alignment was created using CLUSTALW, and MEGA version 4.1 was used to assess the similarities among the aligned sequences using a neighbor joining (NJ) algorithm. A phylogenetic tree based on the deduced amino acid sequences was constructed using a NJ algorithm, and the reliability of the branching was tested using bootstrap re-sampling with 1000 pseudo-replicates.

2.3. Real-time RT-PCR analysis

Total RNA was prepared from cells or olive flounder tissues, using TRIzol® reagent (Invitrogen) according to the manufacturer's instructions. Total RNA concentration was quantitatively determined, and 1 μg of total RNA was used for reverse transcription. The first-strand cDNA was synthesized with random primers using an Advantage® RT-for-PCR kit (BD Sciences). Quantitative real-time PCR was performed using LightCycler® FastStart DNA Master SYBR Green I (Roche) and the following forward and reverse primers: eIF5A-F (5'-AGC CGG AGA GGA GAT TCT GA-3'), eIF5A-R (5'-GCC AAA GGT AGG AAG GTG GG-3'), b-actin-F (5'-TGA GAC TTT CAA CAG CCC TG-3'), b-actin-R (5'-ATC TCC TGC CGT AAG TCC AC-3'), HSP70-F (5'-GAG GCA CAC GAT TAC AGC AA-3'), HSP70-R (5'-TGT TAT TAG TAC ACC TCC TC-3'), VHSV G-F (5'-AGA TGA GGG GAG CCA CAC AC-3'), VHSV G-R (5'-GGG ATG ATC AAT TGG TCC CC-3'), 18 S rRNA-F (5'-ATG GCC GTT CTT AGT TGG TG-3'), 18 S rRNA-R (5'-CAC ACC ATG CTC TAC GTA-3'). Following an initial 10-min Taq activation step at 95 °C, 40 cycles of LightCycler PCR was conducted under the following cycling conditions: 95 °C for 15 s, 55 °C for 5 s, 72 °C for 20 s, and fluorescent reading. Immediately after the PCR, the machine performed a melting curve analysis by gradually (0.1 °C/s) increasing the temperature from 65 °C to 95 °C, with a continuous registration of changes in fluorescent emission intensity. The levels of each transcript were quantified by expressed relative to the 18S rRNA transcript level.

2.4. Cell culture

HINAE olive flounder embryonic cell lines were maintained in Leibovitz L-15 medium with 10% (v/v) heat-inactivated fetal bovine serum (FBS; Gibco BRL) and 1% (v/v) penicillin–streptomycin (PS; Gibco BRL) at 20 °C.

2.5. Cell viability assay (MTT)

The cell viability in the presence or absence of GC-7 was measured by MTT assay (Mosmann, 1982). HINAE cells were plated into a 96-

---

Fig. 1. Nucleotide and deduced amino acid sequences of olive flounder eIF5A cDNA. Start and stop codons are indicated by bold letters. The conserved 12-amino acid hypusine core region (aa 45–56) is in bold and italics, and Lys51 is shown in the box. The polyadenylation signal sequences (AATAAA) are underlined.
well plate at equal density (2.5 × 10^3 cells) in L-15 medium. In 24 or 48 h after infection with VHSV and treatment of GC-7, the cells were treated with 50 µL of MTT solution (5 mg/mL) in serum free medium and incubated for 3 h. Then, the wells were rinsed with PBS and 200 µL of DMSO/well was added to dissolve insoluble purple formazan products. Read the plate on a plate reader (Perkin Elmer) using 550 nm as test wavelength and 630 nm as the reference wavelength. The results were expressed as a percentage of the absorbance of control cultures and all experiments were performed in triplicate.

2.6. VHSV preparation

VHSV was isolated from olive flounder farmed in the Republic of Korea and was propagated in HINAE cells at 20 °C. When the cytopathic effect (CPE) was complete, the supernatants from the infected cell cultures were clarified by centrifugation and stored as aliquots at −80 °C. The virus titer in cell culture supernatants (TCID50/mL) was determined using the end-point dilution method in 96-well plates (Kasai and Yoshimizu, 2001).

2.7. VHSV infection experiments

For the infection experiments, 2 × 10^5 HINAE cells were infected with a dose of 1 × 10^6 TCID50 VHSV for 0, 8, 24, and 48 h. For drug treatments, 2 × 10^5 HINAE cells were infected with a dose of 1 × 10^6 TCID50 VHSV for 1.5 h. After infection, the cells were rinsed twice with PBS without Ca^{2+} and Mg^{2+} to remove the remaining VHSV particles in the medium. Then the cells were further cultured in medium containing various concentrations of GC-7 for 24 or 48 h or in medium supplemented with PBS as a control, to calculate the inhibition of viral protein expression. Experimental challenges were conducted on 100 fish (approximately 12–15 cm in body length) with a dose of 1 × 10^6 TCID50 VHSV administered by immersion at 16 °C. After 1.5 h of infection, the virus was removed by changing the water in the tank. Tissues were taken from three fish at 9, 24, 48, and 72 h after infection. Cumulative mortality was monitored daily over three weeks (data not shown).

3. Results

3.1. Isolation of the eIF5A cDNA of olive flounder Paralichthys olivaceus

We isolated eIF5A cDNA from the EST analysis of a cDNA library of the larval olive flounder Paralichthys olivaceus. The eIF5A cDNA (GenBank accession number F390056) was 1385 bp long and consisted of a 5′ UTR of 54 bp, an open reading frame of 468 bp, and a 3′ UTR of 863 bp containing the polyadenylation sequence (Fig. 1). The eIF5A cDNA encodes 155 amino acids with a theoretical mass of 17.05 kDa. A BLASTP (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) search revealed that the deduced primary sequence of eIF5A contained a well-conserved 12-amino acid hypusine core region (aa 45–56).

Fig. 2. Multiple sequence alignment of flounder eIF5A and those of other known species using ClustalW. Identical residues are indicated by asterisks (*); conservative substitutions are shown by dots (•). High similarity region including 12-amino acid hypusine core region is boxed. Acidic residues in the N-terminal part are shaded.

respectively, and then declined to 1.5-fold at 34 dph (Fig. 4B). The expression of eIF5A mRNA was detected from 7 dph, increased to 3.4- and 3.3-fold at 21 and 27 dph, respectively, and then declined to 1.5-fold at 34 dph (Fig. 4B). We investigated the expression level of eIF5A mRNA during larval stage at brain, liver, intestine, stomach, kidney, spleen, and gill (Fig. 4A). We detected highly in muscle, skin, heart, and gill, moderate in liver and eye, and lower in intestine. Interestingly, flounder eIF5A along with the salmon homologues is more closely related to the mammalian counterparts than to the eIF5A of zebrafish.

3.3. Expression of flounder eIF5A mRNA

To examine the tissue distribution of eIF5A mRNA, real-time RT-PCR was performed using various flounder tissues. eIF5A mRNA was detected highly in muscle, skin, liver, eye and heart, and moderately in brain, liver, intestine, stomach, kidney, spleen, and gill (Fig. 4A). We investigated the expression level of eIF5A mRNA during larval stage at 7, 14, 21, 27, and 34 days post-hatching (dph), eIF5A mRNA was detected from 7 dph, increased to 3.4- and 3.3-fold at 21 and 27 dph, respectively, and then declined to 1.5-fold at 34 dph (Fig. 4B).

3.4. Effect of VHSV infection in the expression of eIF5A mRNA

Real-time RT-PCR during the course of VHSV infection in HINAE cells or flounder kidney revealed that the expression of eIF5A mRNA was affected by viral infection (Fig. 5). In HINAE cells, the expression of eIF5A mRNA was increased 1.35-fold at 8 h post infection (pi), started to decrease from 24 h pi, and decreased to 20% of the initial level at 48 h pi, at which time cells showed cytopathic effects. In flounder kidney infected with VHSV, the expression of eIF5A mRNA was increased 1.4-fold at 9 h pi and then decreased to 35% of the initial level at 24 h pi, similar to the expression profile in HINAE cells. We also measured the expressions of a-actin and HSP70 during the course of VHSV infection in HINAE cells or flounder kidney. The expression of a-actin in HINAE cells was increased 3.75-fold at 8 h pi and then decreased to 20% of the initial level at 24 h pi, similar to the expression profile of eIF5A, but its expression in kidney was decreased during infection. The expression of HSP70 in HINAE cells was increased 4.5-fold at 48 h pi but its expression in kidney was decreased during infection.

3.5. Effect of an inhibitor of eIF5A hypusination in the expression of VHSV G protein

We examined whether the treatment of infected cells with N-guanylated-1,7-diaminoheptane (GC-7), a potent inhibitor of eIF5A hypusination (Liao et al., 1998; Shi et al., 1996), affected the expression of VHSV G protein in HINAE cells. HINAE cells were exposed to VHSV for 1.5 h, rinsed to remove the remaining virus, and further cultured in the presence of several dose of GC-7 (in PBS) or in medium supplemented with PBS. Real-time PCR revealed that GC-7 inhibited the expression of VHSV G protein in a dose-dependent manner, reaching inhibition rates of over 90% at 24 and 48 h pi (Fig. 6A). There were no significant differences in cell viability between untreated and GC-7-treated cultures (Fig. 6B).

4. Discussion

Flounder eIF5A showed a high degree of sequence identity (62.6-88.4%) with eIF5As from mammals, including human, fish such as salmon and zebrafish, and invertebrates. The conservation of the eIF5A sequence has been demonstrated in eukaryotes, including insect (van Oers et al., 1999). Sequence identity is especially high in the region surrounding 12-amino acid hypusine core region in the N-terminal domain (boxed in Fig. 2), which is important for the interaction of eIF5A with the modification enzymes or with its binding partners (proteins or RNA) (Wolff et al., 2007). High content

Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>Accession number</th>
<th>Identity (%)</th>
<th>Amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmon Salmo salar eIF5A</td>
<td>AKK70928</td>
<td>88.4</td>
<td>155</td>
</tr>
<tr>
<td>Mouse Mus musculus eIF5A</td>
<td>CA935154</td>
<td>86.6</td>
<td>149</td>
</tr>
<tr>
<td>Cow Bos taurus eIF5A</td>
<td>NP_00103658</td>
<td>85.7</td>
<td>154</td>
</tr>
<tr>
<td>Horse Equus caballus eIF5A</td>
<td>XP_001018173</td>
<td>85.2</td>
<td>164</td>
</tr>
<tr>
<td>Human Homo sapiens eIF5A</td>
<td>AAD14095</td>
<td>83.1</td>
<td>154</td>
</tr>
<tr>
<td>Frog Xenopus laevis eIF5A</td>
<td>NP_0010080536</td>
<td>81.8</td>
<td>154</td>
</tr>
<tr>
<td>Chicken Gallus gallus eIF5A</td>
<td>NP_990863</td>
<td>80.4</td>
<td>153</td>
</tr>
<tr>
<td>Zebrafish Danio rerio eIF5A</td>
<td>NP_998350</td>
<td>79.4</td>
<td>155</td>
</tr>
<tr>
<td>Silkworm Bombyx mori eIF5A</td>
<td>NP_003137538</td>
<td>65.8</td>
<td>160</td>
</tr>
<tr>
<td>Shrimp Penaeus monodon eIF5A</td>
<td>ABI30653</td>
<td>62.6</td>
<td>157</td>
</tr>
</tbody>
</table>

Fig. 3. Phylogenetic tree depicting the evolutionary relationships among the various eIF5As. The sequences were extracted from GenBank: shrimp Penaeus monodon (GenBank accession number, ABI30653), silkworm Bombyx mori (NP_001037538), zebrafish Danio rerio (NP_998350), salmon Salmo salar (ACH70928), frog Xenopus laevis (NP_0010080536), chicken Gallus gallus (NP_990863), mouse Mus musculus (CAI35154), cow Bos taurus (NP_001003658), horse Equus caballus (XP_001018173), and human Homo sapiens (AAD14095).
of acidic residues were found in the N-terminal extension of flounder eIF5A like some eukaryotic ones, which contribute to signaling this protein for nuclear localization (Parreiras-E-Silva et al., 2007). Consistent with the previous observation (Jao and Chen, 2002), flounder eIF5A fused to green fluorescence protein (GFP) localizes to both the cytoplasm and nucleus in HINAE cells (unpublished data).

Flounder eIF5A mRNA was constitutively expressed in the various tissues and was increased during the developmental stage. These results were consistent with the expression patterns of human eIF5A, which was constitutively expressed at high levels in various human organs (Bevec et al., 1994). In HINAE cells and kidney infected with VHSV, the slight increase of eIF5A mRNA was detected when cells or tissue showed no cytopathic effects (CPE) and then the huge reduction of eIF5A mRNA was observed at 24 h pi when CPE started to appear. The similar expression patterns were observed in the previous reports. The infection of worm S. frugiperda cells with a baculovirus greatly decreased the number of eIF5A transcripts at 12 h after infection, suggesting that eIF5A was an essential protein in insect species and that depletion of this factor towards the end of a baculovirus infection was likely to reduce cell viability (van Oers et al., 1999). During the period of white spot syndrome virus infection in shrimp Penaeus monodon, eIF5A expression was initially increased when no gross signs of disease were visible, but down-regulated compared to uninfected control as the shrimp approached death (Phongdara et al., 2007). The reduced amount of flounder eIF5A mRNA by VHSV infection may cause the reduction of eIF5A protein and that depletion of eIF5A towards the end of a VHSV infection was likely to reduce cell viability (Gosslau et al., 2009; van Oers et al., 1999). eIF5A mediates important cellular processes like cell viability and senescence through its effects on the stability of certain mRNAs related to the regulation of telomere silencing and shortening (Schrader et al., 2006). Although it has been unknown the mechanisms underlying the expression of eIF5A mRNA regulated by viral infection, the reduction of flounder eIF5A mRNA by VHSV infection seems to be a specific phenomenon since the expression pattern of β-actin or HSP70 is different from the pattern of eIF5A.

![Fig. 4. Expression of eIF5A mRNA in the various tissues (A) and during developmental stage (B). Real-time RT-PCR was performed with equal amounts of total RNA isolated from the tissues of normal flounder. 18S rRNA was used as an internal control. Br, brain; M, muscle; L, liver; I, intestine; St, stomach; K, kidney; Sk, skin; F, fin; Sp, spleen; G, gill; E, eye; H, heart. The levels of transcript were quantified by expressed relative to the 18S RNA transcript level. Expression levels were calculated relative to the level of eIF5A in brain (A) or 7 dph (B) of normal flounder is arbitrarily defined as 1.](image)

![Fig. 5. Expression of eIF5A mRNA with the infection of VHSV in HINAE cells (A) and flounder kidney (B). Real-time RT-PCR was performed on equal amounts of total RNA isolated from cells or tissues infected with VHSV (1 × 10⁶ TCID₅₀) for the indicated times. The levels of transcript were quantified by expressed relative to the 18S RNA transcript level. Expression levels were calculated relative to the level of eIF5A in uninfected HINAE cells (A) or normal kidney (B) is arbitrarily defined as 1.](image)
There is still no direct evidence that fish eIF5A is hypusinated like mammal eIF5A. Nevertheless, given the high similarity between the amino acids sequences of flounder eIF5A and human eIF5A, we hypothesized that flounder eIF5A and its hypusination may be involved in the expression of VHSV G protein in cells, similar to the involvement of human eIF5A in the expression of HIV p24 antigen. In human, eIF5A is critical for function of HIV Rev and results in the expression of the viral structural proteins (Ruhl et al., 1993). Treatment with GC-7, a potent inhibitor of eIF5A hypusination, inhibited the expression of VHSV G protein in a dose-dependent manner in HINAE cells, implying hypusine modification of flounder eIF5A and its effect on viral protein expression. Further study will be required to reveal the specific mechanism of the action in relation to the involvement of eIF5A in the expression of VHSV G protein.

In conclusion, we isolated the cDNA for the hypusine-containing protein eIF5A from olive flounder Paralichthys olivaceus. Sequence and phylogenetic analyses revealed that olive flounder eIF5A was more closely related to the eIF5A of salmon than to those of other species. Nevertheless, given the high similarity between the amino acids sequences of flounder eIF5A and human eIF5A, we hypothesized that flounder eIF5A and its hypusination may be involved in the expression of VHSV G protein in cells, similar to the involvement of human eIF5A in the expression of HIV p24 antigen. In human, eIF5A is critical for function of HIV Rev and results in the expression of the viral structural proteins (Ruhl et al., 1993). Treatment with GC-7, a potent inhibitor of eIF5A hypusination, inhibited the expression of VHSV G protein in a dose-dependent manner in HINAE cells, implying hypusine modification of flounder eIF5A and its effect on viral protein expression. Further study will be required to reveal the specific mechanism of the action in relation to the involvement of eIF5A in the expression of VHSV G protein.

In conclusion, we isolated the cDNA for the hypusine-containing protein eIF5A from olive flounder Paralichthys olivaceus. Sequence and phylogenetic analyses revealed that olive flounder eIF5A was more closely related to the eIF5A of salmon than to those of other species. Nevertheless, given the high similarity between the amino acids sequences of flounder eIF5A and human eIF5A, we hypothesized that flounder eIF5A and its hypusination may be involved in the expression of VHSV G protein in cells, similar to the involvement of human eIF5A in the expression of HIV p24 antigen. In human, eIF5A is critical for function of HIV Rev and results in the expression of the viral structural proteins (Ruhl et al., 1993). Treatment with GC-7, a potent inhibitor of eIF5A hypusination, inhibited the expression of VHSV G protein in a dose-dependent manner in HINAE cells, implying hypusine modification of flounder eIF5A and its effect on viral protein expression. Further study will be required to reveal the specific mechanism of the action in relation to the involvement of eIF5A in the expression of VHSV G protein.

In conclusion, we isolated the cDNA for the hypusine-containing protein eIF5A from olive flounder Paralichthys olivaceus. Sequence and phylogenetic analyses revealed that olive flounder eIF5A was more closely related to the eIF5A of salmon than to those of other species. Nevertheless, given the high similarity between the amino acids sequences of flounder eIF5A and human eIF5A, we hypothesized that flounder eIF5A and its hypusination may be involved in the expression of VHSV G protein in cells, similar to the involvement of human eIF5A in the expression of HIV p24 antigen. In human, eIF5A is critical for function of HIV Rev and results in the expression of the viral structural proteins (Ruhl et al., 1993). Treatment with GC-7, a potent inhibitor of eIF5A hypusination, inhibited the expression of VHSV G protein in a dose-dependent manner in HINAE cells, implying hypusine modification of flounder eIF5A and its effect on viral protein expression. Further study will be required to reveal the specific mechanism of the action in relation to the involvement of eIF5A in the expression of VHSV G protein.

In conclusion, we isolated the cDNA for the hypusine-containing protein eIF5A from olive flounder Paralichthys olivaceus. Sequence and phylogenetic analyses revealed that olive flounder eIF5A was more closely related to the eIF5A of salmon than to those of other species. Nevertheless, given the high similarity between the amino acids sequences of flounder eIF5A and human eIF5A, we hypothesized that flounder eIF5A and its hypusination may be involved in the expression of VHSV G protein in cells, similar to the involvement of human eIF5A in the expression of HIV p24 antigen. In human, eIF5A is critical for function of HIV Rev and results in the expression of the viral structural proteins (Ruhl et al., 1993). Treatment with GC-7, a potent inhibitor of eIF5A hypusination, inhibited the expression of VHSV G protein in a dose-dependent manner in HINAE cells, implying hypusine modification of flounder eIF5A and its effect on viral protein expression. Further study will be required to reveal the specific mechanism of the action in relation to the involvement of eIF5A in the expression of VHSV G protein.

In conclusion, we isolated the cDNA for the hypusine-containing protein eIF5A from olive flounder Paralichthys olivaceus. Sequence and phylogenetic analyses revealed that olive flounder eIF5A was more closely related to the eIF5A of salmon than to those of other species. Nevertheless, given the high similarity between the amino acids sequences of flounder eIF5A and human eIF5A, we hypothesized that flounder eIF5A and its hypusination may be involved in the expression of VHSV G protein in cells, similar to the involvement of human eIF5A in the expression of HIV p24 antigen. In human, eIF5A is critical for function of HIV Rev and results in the expression of the viral structural proteins (Ruhl et al., 1993). Treatment with GC-7, a potent inhibitor of eIF5A hypusination, inhibited the expression of VHSV G protein in a dose-dependent manner in HINAE cells, implying hypusine modification of flounder eIF5A and its effect on viral protein expression. Further study will be required to reveal the specific mechanism of the action in relation to the involvement of eIF5A in the expression of VHSV G protein.

Acknowledgments

We thank Hyungtaek Jung for comments on the writing. This work was supported by a grant from the National Fisheries Research and Development Institute (NFRDI), Republic of Korea.

References


