Thesis for the Degree of Master of Fisheries Science

Molecular Cloning of Rod Opsin Gene from Olive Flounder *Paralichthys olivaceus*, Japanese Eel *Anguilla japonica*, Common Carp, *Cyprinus carpio*

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넙치 *Paralichthys olivaceus*, 뱀장어 *Anguilla japonica*, 잉어 *Cyprinus carpio* 로부터 Rod Opsin 유전자의 클로닝

Advisor: Prof. Jong-Myoung Kim

By

Sung Wan Kim

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Abstract

Rhodopsin, a dim-light receptor, has been extensively used as a model system to study of G Protein-coupled receptor transmitting extracelluar signals such as neurotransmitters, hormones, odorants and light. Fish living under various photic environments posses visual systems adapted to the habitats. To study the molecular mechanism of spectral tuning mechanism in fish, rod opsin genes of olive flounder *Paralichthys olivaceus,* Japanese eel *Anguilla japonica*, and common carp *Cyprinus carpio*, were isolated. Full-length opsin genes of *P. olivaceus*, *A. japonica*, *C. carpio* were obtained by PCR amplification of genomic DNA. Sequence analysis of the rod opsin gene reveals of 1056 bp opsin genes encoding 352 amino acids in olive flounder and Japanese eel and 1062 bp encoding 354 a.a in common carp. The deduced amino acids showed typical feature of rod opsin, such as Schiff's base formation (K296) and its counterion (E113) and two cysteines forming disulfide bond(C110 and C187). However one cysteine involved in palmitoylation is replaced by Phe in Japanese eel. The sequence alignment of other fish rod opsin shows the similarity between *P. olivaceus* and *Hippoglossus hippoglossus* (94%), *A. japonica* and *A. anguilla* (98%), *C. carpio* and *C. auratus* (95%).

Figure legends

Table legends

Molecular Cloning of Rod Opsin Gene from Olive Flounder *Paralichthys olivaceus*, Japanese eel *Anguilla japonica*, and Common Carp, *Cyprinus carpio*

Ⅰ. Introduction

G protein coupled receptors (GPCRs), one of the largest membrane receptor families play important role in transmitting outside signals into inside (reviewed by Khorana et al., 2002). GPCRs respond to diverse signal such as neurotransmitters, hormones, light, smells, and tastants. Upon recognition of the stimuli, GPCRs initiate conformational change followed by a signal transduction cascade through GTP/GDP-binding proteins. Although GPCRs recognize such a diverse array of signals, all GPCRs have a common structural topology, seven transmembrane helices. This, together with some conserved sequences, suggests a common activation mechanism in GPCRs.

There are two type of photoreceptor cells in most vertebrates. Rod cell is responsible for scotopic vision and the cone cell is responsible for photopic vision (Khorana, 2000). Rhodopsin, a dim -light photoreceptor, is composed of an opsin forming a seven transmembrane region and retinal chromophore. Absorbtion of a photon by rhodopsin cause isomerization of 11-cis-retinal to all-trans form. This induces a

series of conformational changes in the protein initiating intracellular signaling. Rhodopsin has been used as a model system for the study of GPCRs because of its higher expression level and its easier accessibility for bio-physical analysis.

Fish living under various photic environments were known to posses the visual systems adapted to their habitats. Absorption maxima of photoreceptor of fish are considered to be closely related the ambient light conditions (Lythgoe, 1979; Hunt et al., 1996). American and European eels were known to change the pattern of their expression when the eel migrate from a river to the deep sea to adapt the new photic environments (Acher et al., 1995; Hope et al., 1998). It was reported that a change in the primary amino acid structure of the opsin cause a shift of their absorption maximum (Yokoyama, 1995). This suggests that fish living under the different photic environments and temperature is a good model system to study the molecular basis of visual photoreceptors. Olive flounder lives in benthic environments after spending the fry stage in the pelagic zone. Japanese eel spawn in the sea. The small eels ascend the rivers in schools and develop and grow in freshwater. Common carp is inhabited in freshwater. They were found in the water in which the temperature range between 3 and 35°C. They were hardy and tolerant of a wide variety of conditions but generally favor large water bodies with slow flowing or standing water and soft bottom sediments. Common carp could also thrive in large turbid rivers. Therefore, there might possess opsins with different absorption maximum and protein structure.

Ⅱ. Materials and Methods

1. Materials

T4 DNA ligase and AccuPreP® Genomic DNA extraction kit were purchased from Bioneer Corporation (Daejeon, Korea). Wizard® Plus Maxipreps DNA Purification System was purchased from Promega Corporation (Madison, WI). Various restriction endonucleases were purchased from Bioneer (Daejeon, Korea) and New England Biolabs (Beverly, MA), calf intestine endonucleases were also obtained from New England Biolabs (Beverly, MA). Plasmid Purification mini Kit, Gel Extraction Kit and PCR Purification Kit were purchased from NucleoGen (Seoul, Korea). DNA Walking SpeedUp™ PremixKitⅡ was obtained from Seegene (Seoul, Korea). pGEM®-T Easy Vector system was purchased from Promega Corporation (Madison, WI). Oligonucleotides and 5×HiQ-PCR mix were obtained from Genotech (DaeJeon,Korea). PCR primers are listed in the Table 1. MyCycler™ Thermal Cycler used for PCR reaction was obtained from Bio-Rad Laboratories (Hercules,CA).

Table 1. List of sequence of oligonucleotides used for the experiment

primer name	sequence (5'→3')	comment
OpsinF1	GCA AGA ATT CAT GAA CGG CAC AGA GGG ACC	Rod opsin
OpsinR1	ATT TGC GGC CGC TTA TGC TTA TGC AGG GGA CAC AGA G	
HCBOpF1	CGA GAG GTG GAT GGT TGT C	DNA walking PCR
HCBOpF2	C/ TGA GAC CAC CCA GAG GGC	
HCBOpF3	TGC CAG CGT GGC CTG GTA	
HCOpR1	GAC AAC CAT CCA CCT CTC G	
HCOpR2	CGA CGT GTA CAT CGT CGT GGT G	
HCOpR3	GGA AGA ACA TGT ACG CGG CCA GG	
OpsBgF1	GAA GGC ATG CAG TGT TC	
HCBOpF2	C/ TGA GAC CAC CCA GAG GGC	
HCBOpF3	TGC CAG CGT GGC CTG GTA	
OpsBgR1	GAA CAC TGC ATG CCT TC	
BgOpR2	CGA CGT GTA CAT CGT AGT GGT G	
BgOpR3	GCG TGA ACA TGT AGG CAG CCA GG	
OpsF1	GAG GGC ATG CAG TGC TC	
TSPFCF2	TGT GCT GTC AAG GAG GCT GC	
TSPFCF3	GTG TGG CCT GGT ATA TCT TC	
OpsFCR1	GAG CAC TGC ATG CCC TC	
TSPFCR2	ACC GCA AGG TTC AGA AGG ATG	
TSPFCR3	GAA ACA TAT AGG CAC CCA GG	

2. Genomic DNA extraction and cloning of rod opsin gene

2-1. Isolation of Genomic DNA

Genomic DNA of fish was isolated from their blood by using AccPrep®Genomic DNA Kit according to manufacturer's instructions. 20uL of ProteinaseK used to lysis of cells was added 80 uL of blood in 1.5 ml microcentrifuge tube. After the addition of 120 uL of PBS [10 mM Tris-Cl, 25 mM EDTA, 150 mM NaCl], 200 uL of Binding buffer (GC) was added and mixed by vortex immediately. This was followed by incubation at 60℃ for 10 min. The suspension was mixed well by pippeting after addition of 100 uL of isopropanol. Upon centrifugation for 1 minute, the lysate was carefully transferred into the upper reservoir of the binding column tube (fit in a 2 ml tube) without wetting the rim. The tube was then centrifuged at 13000 rpm until the lysate completely passed the binding column. For filtration, the binding column was transferred to a new 2 mL tube, washed 500 uL of Washing buffers 1 (W1) and 2 (W2) by using a centrifugation at 13000 rpm for 1 min, respectively. After the centrifugation at 13000 rpm for 1 min to remove ethanol, the binding column was transferred to a new microcentrifuge tube. Upon addition of 200 uL of distilled water, tube was centrifuged again at 13000 rpm for 1 min for the elution.

DNA was confirmed by 1% agarose gel electrophoresis followed by staining with ethidium bromide (0.5 ug/ mL). DNA was stored at -20℃ until its further use.

2-2. Amplification of opsin gene by using PCR

Oligonucleotides(F1: 5'-GCA AGA ATT CAT GAA CGG CAC AGA GGG ACC-3', and Opsin R1: 5'-ATT TGC GGC CGC TTA TGC TTA TGC AGG GGA CAC AGA G-3') designed from conserved regions of zebrafish, Japanese medaka, Atlantic salmon opsin genes (Philp et al., 2000) were used for rod opsin gene amplification. Moreover, *EcoR* Ⅰ, *Not* Ⅰ recognition sequences were included in Opsin F1, R1 oligonucleotides, respectively, at their 5' ends to facilitate the cloning into the corresponding site of pMT4 (Oprian et al., 1987). PCR amplification was carried out with 3 uL of genomic DNA(0.1 ug/ul), 2 uL Opsin F1, R1 primer, 10 uL of 5×HiQ-PCRmix in 50 uL reaction volume.

Reaction was carried out with an initial denaturation at 95℃ for 5 min, together with 30 cycles of reactions comprised of denaturation at 94℃ for 1min, annealing either at 48℃ (common carp) or 50℃ (olive flounder, Japanese eel) for 1min, and extension at 72℃ for 3min followed by final extension at 72℃ for 10 min. Analysis of the PCR product was carried out with agarose gel electrophoresis. The PCR product of 1 kb in size was purified by gel extraction followed by ligation into pGEM-T easy vector (50 ng/ul) using Promega's pGEM-T easy vector kit. For this, the purified PCR product mixed with T-vector and 2× ligation buffer were incubated for 1 hour at 25℃. The Recombinant DNA was transformed into *E.coli*, DH5α.

2-3. Transformation of *E.coli* by using recombinant plasmid

2-3-1. Preparation of Competent Cells

Competent cells were prepared by using Inoue method. For this, 1/200 volume of *E.coli* grown previously at 37℃ were inoculated in 200 mL LB medium for further growing the cells overnight at 22℃ with moderate shaking (180 rpm). When the OD_{600} reached 0.6, the culture was chilled on ice for 10 minutes. Cells were harvested by centrifuging with 2,500g, at 4℃ for 10 minutes and then resuspended in 40 mL of ice-cold transformation buffer (TB)[10 mM Hepes, 55 mM $MnCl_2$, 15 mM $CaCl_2$, 205 mM KCl, pH 6.7]. Upon incubation on ice for 10 min, cells were harvested by centrifugation at 2,500g for 10 min at 4℃. Cells resuspended in 20 mL of TB buffer were added with 1.5 mL of DMSO to the final concentration of 7% followed by incubation on ice for 10 min. The cells divided into 1.5 mL tube were immersed in liquid nitrogen and stored at -80℃until its use.

2-3-2. Transformation of recombinant plasmid into the *E. coli*

For the transformation of *E. coli* using the recombinant DNA, ligated DNA was incubated with 100 uL of the competent cell on ice for 30 min. Heat shock of the cell was carried out at 42℃ for 1 minute. Upon incubation on ice for 3 minutes followed by the addition of 1 mL LB medium, cells were incubated at 37℃ for 1 hr. Cells transferred onto

LB agar plate containing the Ampicillin (100 ug/mL) together with 2% X-gal depending on the vector used for the experiment were incubated overnight at 37℃.

2-4. Identification of recombinant DNA

The bacterial colonies selected from transformants were inoculated and incubated in LB medium containing ampicillin (100 ug/ mL) at 37℃ for overnight. Plasmid DNA was isolated by using the Plasmid Purification Kit according to manufacturer's instructions. For this, 1.5 ml of the culture transferred into microcentrifuge tube was harvested by centrifugation at 13,000 rpm for 1 min. Cells were resuspended in 250 uL of Resuspension Solution. Cell lysate was prepared by the addition of 250 uL of Lysis Solution. The content was mixed gently by several times of inversion and incubated until the cell suspension clears, approximately for 5 min. 300 ul of Neutralization Solution was added into tube and mixed by several times of inversion. Upon centrifugation at 13,000 rpm for 10 min, the supernatant was transferred into the spin column supplied by the manufacturer and then centrifuged at 13000 rpm for 1 min. To wash the column, 750 uL of Washing A solution was added to column, and then centrifuged twice at 13,000 rpm for 1min. DNA was eluted with 50 uL of distilled after the centrifugation at 13,000 rpm for 1 min. Plasmid DNA were stored at -20℃until further use.

Restriction endonuclease digestion was carried out according to the

manufacturer's suggestion. *EcoR* Ⅰ restriction endonucleases was used to identify the recombinant vector. Plasmids digested with restriction endonucleases were subject to by 1% agarose gel electrophoresis. DNA sequence in the plasmid were confirmed by DNA sequencing analysis.

2-5. Cloning of the 5'- and 3'-end of the rod opsin gene

To obtain the regions encoding the 5'- and 3'-end of the opsin gene, the regions flanking its 5'- and 3'-ends were amplified by using DNA walking speedUP™ Premix Kit Ⅱ. Fifteen primers were designed to perform the experiment. Primers corresponding to the anti-sense strands of opsin gene from common carp (HCBOpF1, HCBOpF2, HCBOpF3), Japanese eel (OpsBgF1, OpsBgF2, OpsBgF3), and oliver flounder (OpsF1, TSPFCF2, TSPFCF3) were designed. Oligonucleotides corresponding to the sense strands of opsin gene from common carp (HCOpR1, HCOpR2, and HCOpR3), Japanese eel (OpsBgR1, BgOpR2, and BgOpR3), and oliver flounder (OpsFCR1, TSPFCR2, and TSPFCR3) were also included (Table 1).

DNA walking PCR was performed with universal primers supplied by DNA walking speedUP™ Premix Kit together with the template specific primers. Reaction was carried out according to manual provided by the manufacturer in 20 ul of reaction containing 2 uL genomic DNA(0.1 ug/ul), 1 uL (10 uM) of template specific primer, 2 uL of 5 uM DW2-ACP primer (supplied, DW2-ACP 1, 2, 3 and 4), and 10 uL

of 2 × SeeAmp^{TM}ACP^{TM}Mastermix Ⅱ to amplify the regions flanking the 3' and 5'-ends of opsin genes. The first PCR was conducted under the following condition : initial denaturation at 94℃ for 5 min, an annealing at 42℃ for 1 min, and extension at 72℃ for 2 min, 30 cycle of amplification including denaturation at 94℃ for 30 sec, annealing at 55℃ for 30 sec, extension at 72℃ for 100 sec and final extension at 72℃ for 10 min. The first PCR product was purified using the PCR Purification Kit.

The second PCR was carried out with 2 uL of purified first PCR product as template, 1 uL of designed second primer(10 uM), 2 uL of 5 uM DW2-ACPN, 5 uL of distilled water, 10 uL of 2 × SeeAmp^{TM}ACP^{TM}Mastermix Ⅱ as describe in the manual provided by the manufacturer. Reaction condition was comprised of an initial denaturation at 94℃ for 3min, followed by 35 cycles of the denaturation at 94℃ for 30 sec, annealing 60℃ for 30 sec, extension at 72℃ for 100 sec, and final extension at 72℃ for 7 min.

The third PCR mixture included 2 uL of second PCR product as template, 1 uL(10 uM) of designed third primer, 2 uL of 5 uM UniP2, 5 uL of distilled water, and 10 uL of 2× SeeAmpTMACPTM Master mix Ⅱ. The third PCR reaction was carried out with initial denaturation for 3 min at 94℃, 30 cycle of amplification containing denaturation for 30 sec at 94℃, annealing for 30 sec at 65℃, extension for 80 sec at 72℃, and final extension for 7 min at 72℃. PCR products resolved upon agarose gel electrophoresis were purified by using Gel Extraction Kit and then cloned into the pGEM-T Easy Vector. Recombinant DNA were

transformed into *E.coli* as described above. Transformants selected on LB agar plate containing Ampicillin (100 ug/mL) and 2% X-gal were subject to plasmid isolation. To identify the inserts in recombinant vectors, plasmid DNA isolated from the transformants were subject to restriction digestion and DNA sequencing analysis.

3. Cloning of rod opsin gene into the expression vector

The full length opsin genes of olive flounder, Japanese eel, and common carp were cloned into pMT4 for the expression analysis. This was carried out by amplification of opsin genes with forward and reverse primers (Table 2) containing restriction endonucleases *EcoR* Ⅰ and *Sal* Ⅰ recognition sequences at 5', and 3'-ends of coding region, respectively, for facilitating the cloning into pMT4. The forward primers contain a translation start codon (AUG). Reverse primers were designed to replace the stop codon of the opsin gene with a *Sal* Ⅰ restriction site (replaced by *Xho* Ⅰ in OpsinRXho Ⅰ). This facilitate the cloning of opsin gene fused with a sequence corresponding to a C-terminal bovine rod opsin 1D4 epitope (ETSQVAPA) which would be recognized by the anti-Rho1D4 monoclonal antibody.

PCR reaction was carried out as described above. PCR was conducted by denaturation 95℃ for 5 min, 30 cycle of amplification containing denaturation at 94℃ for 1 min, annealing at 47℃ (for

common carp), at 50℃ (for olive flounder, Japanese eel) for 1 min, extension at 72℃ for 3 min, and final extension at 72℃ for 10 min. The PCR product purified by PCR Purification Kit were digested by *EcoR* Ⅰ and *Sal* Ⅰ. However, opsin gene of common carp were treated with *Xho* Ⅰ instead of *Sal* Ⅰ as the opsin gene of common carp contains *Sal* Ⅰ restriction enzyme site in the coding region. The digested PCR products purified by using Gel Extraction Kit were cloned into the pMT4 digested with *EcoR* Ⅰ and *Sal* Ⅰ followed by calf intestine endonucleases (Oprian et. al., 1987). Recombinant plasmids transformed into *E.coli.* were identified by restriction analysis using *BamH* Ⅰ (in case of olive flounder, Japanese eel), *Hinc* Ⅱ (in case of common carp) restriction endonucleases. Analysis of the constructs were also carried out by DNA sequencing (Genotech, Korea). The obtained DNA sequence was compared with NCBI database using BlastN & BlastX.

Table 2. Sequences of oligonucleotides used to generate the recombinant opsin constructs for expression analysis. Oligonucleotides were used to amplify the opsin genes of common carp (opsinF1 and opsinRXho Ⅰ), olive flounder (opsinewF1, opsinR12, opsinF8MF, and Fg1LR), and Japanese eel (opsinF1 and EEOpsinR1).

primer name	sequence (5'→3')	comment
OpsinF1	GCAAGAATTCATGAACGGCACAGAGGGACC	Full-length of Rod opsin
OpsinRXho Ⅰ	GACCTCGAGGCAGGGGACACGGAGCTGGAA	
OpsinewF1	GCAAGAATTCATGAATGGAACGGAGGGACC	
OpsinR12	GGTGGTCGACGCTGGTGACACGGAGCTGGA	
OpsF8MF	GAATTCATGAATGGAACGGAGGGACCAAATTTTTATGTCCCTATG	
Fg1LR	AGTCGACGCTGGTGACACGGAGCTGGAGGA	
EEOpsinR1	GGTGGTCGACGCGGGAGACACAGAGGACAC	

EcoR Ⅰ (GAATTC), *Sal* Ⅰ (GTCGAC), *Xho* Ⅰ (CTCGAG) site are indicated by underline.

3-1. Large-scale purification of recombinant plasmid

Recombinant plasmid cloned into pMT4 expression vector was prepared in a large scale for the expression in animal cell. For this, culture grown overnight on a small scale was inoculated into 400 mL LB broth medium containing ampicillin (100 ug/ mL) followed by incubation at 37℃ for 12 hours with shaking. Cells harvested by centrifugation at 4000 x g for 10 min. The harvested cell resuspended in 30 mL of Cell Resuspension Solution [50 mM Tris- HCl, 10 mM EDTA, 100 ug/ mL RNase A, pH 7.5] were mixed with 30 mL of Cell Lysis Solution [0.2 M NaOH, 1% SDS] until the solution becomes clear. Upon addition of 30 mL of Neutralization Solution [1.32 M potassium acetate, pH 4.8], the lysate was centrifuged at 6,000 xg for 30 min. The supernatant filtered through gauze into 50 mL tube was mixed with 0.5 volume of isopropanol followed by centrifugation at 13,000 rpm for 30 min. The pellet was dried and then dissolved in 2 ml of distilled water. This was incubated with 10 mL of Wizard® Maxi preps DNA purification Resin and then passed through the column. The column was washed with 25 mL of Column Washing Solution [80 mM potassium acetate, 8.3 mM Tris- HCl (pH 7.5), 40 uM EDTA, approximately 55% ethanol]. The column on top of the 50 mL tube was centrifuged at 2,500 rpm for 5 min in a swinging bucket rotor. The column was dried and placed in a new 50 mL tube. To elute DNA, 2 mL of distilled water was added to the maxi column, and centrifuged at 2,500 rpm for 5min. The eluate was filtered through 0.2 uM Syringe

filter and centrifuged at 13,000 rpm for 1 min to remove the resin. DNA was stored in -20℃.

4. Sequence alignment and phylogenetic analysis

Full-length amino acid sequence of rod opsins were aligned with the other known opsin sequences using ClustalW (Thompson *et.al.*,1994). Accession numbers of opsin amino acid sequence searched from NCBI Gen Bank were Zebrafish (*Danio rerio,* BC045288.1), Japanese medaka (*Oryzias latipes*, AB180742.1), goldfish (*Carassius auratus,* P32309), European eel (*Anguilla anguilla*, deep water form, Q90214, fresh water form, Q90215), White spotted conger (*Conger myriaster*, fresh water form, BAB21487), common sole (*Solea solea*, CAA77254), Flat head mullet (*Mugil cephalus*, CAA77250), Atlantic halibut (*Hippoglossus hippoglossus*, AAM17918), Nile tilapia (*Oreochromis niloticus*, AAY26023), *Labeotropheus fuelleborni* (AAY26028), *Paralabidochromis cyaneus* (AAV93304), human (*Homo sapiens*, P08100), striped red mullet (*Mullus surmuletus*, CAA77248), torafugu (*Takifugu rubripes*, AAF44621), saddle back dolphin (*Delphinus delphis*, AAC12761), dog (*Canis lupus familiaris*, CAA50502), and bare-tailed wolly opossum (*Caluromys philander*, AAQ82903).

Phylogenetic tree was constructed by neighbor-joining method using MEGA v 4.0. Various species of rod opsins exhibiting higher homology with predicted Japanese eel, olive flounder, common carp's rod opsin were selected using the Blastp in NCBI. Assessing tree reliability was tested using a bootstrap with 1000 replicates.

Ⅲ. Results and Discussion

Fish living under various light environments possess photoreceptor cells adapted to their habitats. In addition, fish migrating from the pelagic zone to a benthic habitat, or vice versa, during ontogeny can change their visual receptors depending on their developmental stages (Helvik et al., 2001). Therefore, fish is good model system for studying visual systems adapted to specific light environments.

Rhodopsin, a dim-light photoreceptor in visual signal transduction, has been used as a prototype for the structural and functional study of GPCRs. In order to study molecular characteristics of rod opsin adapted to different photic environments, genomic DNA was first isolated from the whole blood of the fish (Fig. 1). Analysis of genomic DNA using agarose gel electrophoresis indicated the intactness of the DNA. Amplification of rod opsin gene was performed by using PCR. Oligonucleotides F1 and R1 containing conserved sequence corresponding to the 5' and 3' ends, respectively, were designed to amplify rod opsin gene. PCR was carried out with different annealing conditions. The products obtained from annealing temperature at 48℃ (in case of common carp), 50℃ (oliver flounder, Japanese eel) showed approximately 1 Kb of DNA fragments (Fig. 2). The size was similar the size of opsins from other fish searched by using BlastN and BlastX. To acquire the full length of rod opsin gene exactly, DNA walking was carried out with target specific sense or antisense primers based on the

sequence of rod opsin gene obtained from PCR (Fig. 3). Several PCR products were obtained from amplification of the regions flanking the 5'- and 3'-end of the opsin genes. PCR products of 250 bp and 800 bp (olive flounder, Fig. 4), 460bp and 300bp, (Japanese eel, Fig. 5), and 800bp and 850bp (common carp, Fig. 6) corresponding to the 5'- and 3'end of the gene, respectively, were obtained from DNA walking. Full length of opsin genes with the flanking region sequence were acquired and analyzed based upon DNA sequence analysis of the fragments. The complete coding sequence of rod opsin genes consist of 1,056-bp structural gene encoding 352 amino acids (in cases of olive flounder and Japanese eel, Figs. 7 and 8) and 1,062-bp encoding 354 amino acids (common carp, Fig. 9) The predicted open reading frame showed a high homology to rod opsins previously identified. This indicates that the isolated gene from several fishes belong to the opsin group.

Amino acid sequences conserved in various rod opsin and GPCRs were observed by sequence analysis. These include a lysine (K296) residue within the putative transmembrane domain Ⅶ that could attaches to the chromophore by Schiff's base linkage (Wang et al., 1980) and a counterion, glutamic acid (E113) in the predicted third transmembrane domain (Sakmar et al., 1989). Two cysteine residues found in positions Cys-110 and Cys -187 may form a disulfide bridge that is critical for the conformation of functional opsin and GPCRs (Karnik et al., 1988). Two asparagine residues found at positions 2 and 15 may be a glycosylation site important for the targeting and folding of rhodopsin (Kaushal et al., 1994). Several serine and threonine

residues were also found in the carboxyl terminal where the potential phosphorylation might occurs (Ohguro et al., 1994).

The important residues for the activation of rhodopsin (Franke et al., 1990), E134 and R135 are also conserved although the Trp is substituted for Tyr at 136 position. In addition, two cysteine residues, Cys322 and Cys323 are found in C-terminus, which might be required for anchoring rhodopsin in the cell membrane by palmitic acid esterification (Ovchinnikov et al., 1988). However, Cys322 was replaced by Phe322 in Japanese eel.

Several studies have analyzed the amino acid sequence of rod opsins in marine species (Archer et al., 1995) and fresh water species (Hunt et al., 1996) living at different depths. Three amino acids residues (position 83, 261, and 292) in rod opsin have been identified to be important for spectral tuning (for review see Bowmaker & Hunt, 1999). For fishes living near surfaces, typical amino acid residues in the rod opsin tuning positions were known to be asp, tyr, ala or asp, phe, ala. The corresponding amino acid residues in both marine and freshwater species living at depths of 400-5000m were asn, ser, phe. While rod opsins of Japanese eel and olive flounder have amino acids asn, phe, ser, and asn, phe, ala respectively, opsin of common carp has asp, phe, ala in these positions. This implies that Japanese eel and olive flounder are more adapted to the zone above 500m.

Deduced opsin amino acid sequences of olive flounder, Japanese eel, and common carp were compared to those of isolated from various species (Fig. 10). It show the presence of the predicted 7

transmembrane regions. In general, the regions corresponding to 7 transmembrane region are more conserved. The table 3, 4, and 5 show the percent of identity between among rod opsins by using ClustalW program. Opsin of olive flounder showed 94%, 94%, 93%, 85%, and 73% amino acid identity with *Hippoglossus hippoglossus, Solea solea, Pseudopleuronectes americanus, Danio rerio* and *Homo sapiens,* respectively (Thompson et al., 1994). Japanese eel showed 98%, 90%, 85% and 78% amino acid identity with opsin isolated from *Anguilla anguilla, Conger myriaster, Danio rerio*, and *Homo sapiens*. Common carp showed 95%, 92%, 83%, 81%, and 76% identity with opsins isolated from *Carassius auratus, Danio rerio, Astyanax mexicanus, Sardina pilchardus* and *Homo sapiens*. Phylogenetic trees were made using neighbor-joining method (Fig. 11) to analyze the phylogenetic relationships of olive flounder, Japanese eel, and common carp. Various vertebrate opsins ranging from human to teleost were included for comparison. Opsins were grouped into five main branches, reflecting each fish's opsin belong to these five classes. Amino acid residues in opsin may affect the absorption maximum of rhodopsin. Opsin sequences from various organisms and their absorption maxima were studied to determine the relationship between the amino acid sequence and λmax of visual pigments (Nakayama and Khorana , 1991; Imai et al., 1997; Yokoyama and Radlwimmer, 1998). In order to examine the spectral characteristics of rhodopsin in fishes used in the study, genes encoding the full length opsin genes were amplified by using primers OpsinewF1, OpsinR12, OpsinF8MF, Fg1LR, and OpsinR12 (olive flounder), OpsinF1 and

EEOpsinR1 (Japanese eel), OpsinF1 and OpsinRXho Ⅰ (common carp). These contain sequences corresponding to the 5'- and 3'-end of the gene together with the restriction endonuclease site to facilitate the cloning into the expression vector. The λmax of rod opsin protein will be measured by UV/vis spectrometry analysis upon transfection into animal cell.

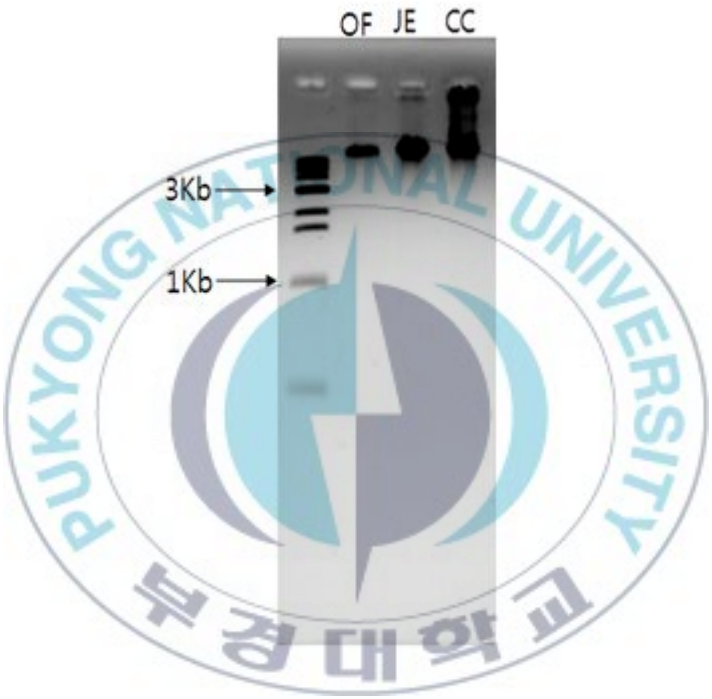

Figure 1. Genomic DNA isolated from whole blood of olive flounder *Paralichthys olivaceus,* (OF), Japanese eel *Anguilla japonica*, (JE), and common carp *Cyprinus carpio*, (CC) were subject to 1% agarose gel electrophoresis. The right lanes (OF, JE and CC) include isolated genomic DNA and the left lane includes KB ladder (Bioneer,Korea).

Figure 2. PCR products obtained from the amplification of genomic DNA using primers corresponding to the conserved sequences of known rod opsin genes from other fish. The right lanes include PCR products obtained from olive flounder (OF), Japanese eel (JE), and common carp (CC). The left lane includes KB ladder (Bioneer, Korea).

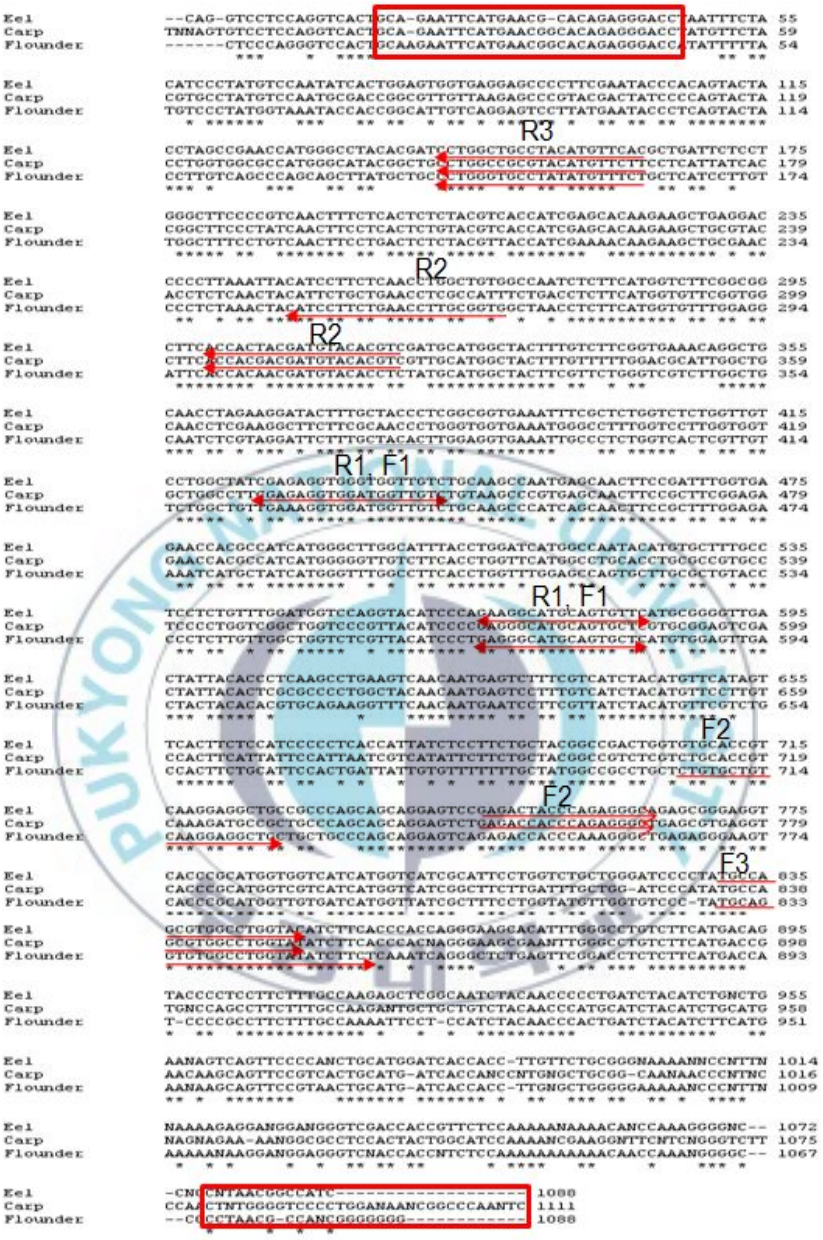

Figure 3. Comparison of the predicted rod opsin genes from olive flounder, Japanese eel, and common carp. Arrow lines indicated the position and sequence of primers used for DNA walking PCR.

<1>

GAGTTTAGGTCCAGCGTCCGTGGGGGGGGACGGTCTCCCTCTTCATCACTATCCTACAC
AGCCAGAAGAAACACCACTGAAGGGCTGATCGCAACCGCAAGCCGCAACC**ATG**AATG
GAACGGAGGGACCATATTTTTATGTCCCTATGGTAAATACCACCGGCATTGTCAGGAG
TCCTTATGAATACCCTCAGTACTACCTTGTCAGCCCAGCAGCTTATGCTGCCCTGGGTG
CCTATATGTTTC

<2>

GTGTGGCCTGGTATATCTTCTCAAATCAGGGCTCTGAGTTCGGACCTCTCTTTATGACCA
TCCCCGCCTTCTTTGCCAAGAGTTCCTCCATCTACAACCCACTGATCTACATCTTCATGA
ACAAGCAGTTCCGTAACTGCATGATCACCACCTTGTGCTGTGGGAAGAATCCCTTTGA
GGAGGAGGAGGGAGCATCCAGCACCAAGACCGAGGCCTCTTCTGCCTCCTCCAGCTCC
GTGTCACCAGCA**TAA**AAAGGGCCATCTACAAAGGCTCTGTCATTCACCATCCAAGAAG
AAGACTTCTGCTCCCCCCGGGAAACGACCGAAGGCTAATCTCTACAGAAATAACTTCC
TTTTTGTATTTTTACGAACAAGTTGGTTCAACCTAAAGACAGTTGCAGGAAAGGTCAGC
CCATTACAGAGTTGTTCCTGTATGTACAAAATATCCAACCTAACAATCTATAATTTTTTT
CCTGAGAGTAAAGGGGAAAAATGTTATCTTTAACAGTTGGATCCTATATCATATTGGCT
TATTTTTGAATGTAGAGGCATGTAATCAAGGCAATGTAAAATAAATCGCACTTTGCAA
ATGACTCATTCTGTTTATGTTTTACTTACAATTTGGGTAGGAAAGTTCATATGACTGTAG
TTTATTTAATCAAATGAATAAATATGAATGACCTTTTCAGAATGCATTTATGTGTTGAGT
CAATTTCTATTTTTGAGATATTGTAGGGACGACCGCCTTTCTACTCAGGCAGATACACA
CAGAATGGTACAAAAAACTTTGACAGTGTGAGACTCAGTCGTGGTACTGCATTGCTAC
TGTAATCTCATTTCAAACACAGGTCACGCCCCCCCCCACGGACGCTGGAACCTAACTC
AATCTCTAGAGGATCCCC

Figure 4. DNA sequence of the fragments corresponding to the 5'-end and 3'-end of opsin gene of olive flounder obtained from DNA Walking. <1> shows 250bp DNA fragment corresponding to the 5'-end region, and bold letter indicates the start codon. <2> shows 800bp DNA fragment corresponding to the 3'-end region of the opsin gene and bold letter indicates the stop codon.

<1>

GAGTTTAGGTCCAGCGTCCGTGGGGGGGGTCGACCTGGTGCAGGATTACCTGGAACAG
AGTGAGAAAGAAAGAGAGAAAAACGGCCCAGAAAGCACTGGGGCTTCCTTATTATAG
GGTTTACCCCCAGGTGCTCTCAATTAGAAAGGGGTGCACCGCACACACTGGCCGACTC
CTGTAACTACAATAAAAATAAAACAATATTAGAGACAGTCGTCACATCTGTTTCCAGT
ATCAGTGCATCACTCTGCTGTTTGAAGTGTAAACACCTCACAGCTAGACGAGACAACA
CTTCTGAAGGACTGATCGAAAAACGCAGCC**ATG**AACGGCACAGAGGGCCCTAATTTCT
ACATCCCTATGTCCAATATCACTGGAGTGGTGAGGAGCCCCTTCGAATACCCACAGTA
CTACCTAGCCGAACCATGGGCCTACACGATCCTGGCTGCCTACATGTTCACGC

<2>

TGCCAGCGTGGCCTGGTATATCTTCACCCACCAGGGAAGCGAATTTGGGCCTGTCTTCA
TGACCGTGCCAGCCTTCTTTGCCAAGAGCGCTGCTGTCTACAACCCATGCATCTACATC
TGCATGAACAAGCAGTTCCG**TAA**CTGCATGATCACCACCCTGTGCTGCGGCAAGAACC
CCTTCGACCCCCCCCACGGACGCTGGACCTAAACTCA

Figure 5. DNA sequence of the fragment flanking to the 5'- and 3'-end of the opsin gene of Japanese eel obtained from DNA Walking. <1> shows 460bp DNA fragment corresponding to the 5'-end region, and bold letter indicates the start codon. <2> shows 300bp DNA fragment corresponding to the 3'-end region, and bold letter indicates the stop codon.

<1>

TGAAAAAGTTTGTGATTCGGTGACATATAAGTGACTTCATTTTGAATCAGTTCAAACTG
AACAAACTTCTCTTGTCAATCTTGCTTTGAAATTTGGTTGTCTCACTTGTGCACTTTCAA
ATGTGTCCAAATCCTATTCGGGGCCTCTGCATTGGATGCTGCTTGATGGAAGTTAAGTC
ATCTTTATTTATATAGTGCTTTTAACAATACAGATTGTGTCTAAGCAGCTTTACAGTATT
AAATAGGAAAATAGTGTGTAATAATGCAAAAGAACAATGGTAAACACGAAATTTTCA
GGTTCACCATTGCTTCAGTGATGTCATCGTACGAGTGTGCCATTGTGTCTCAAATTAAG
TTGTGTGTGTGTGTTTTTTCTTTTCGTTTTTTTCCTATCGCCGTTTACCTGCCTTGTGCA
GGTACAACCTTAATAACTTGATAAGTTGCCAGATATGCAGTGCAATGATGGCTGGGAT
TATTTTATTAGCTGTGGCCTAAATAGACTCGTGCTGACAGCCTGGAAACATCAGGTAAT
CCCAAGCGAGCCTCTATAAAGCGTGGTGCACGCTCGCCCCGTCAAGTCGTAGCACGGT
CCTGCCTCGTTTCTCCACAGTCCTGCCGAGCCATCCAAACACTACTGCAGAAAGGGGC
TGAGCACAACATCCAACCGCAGCC**ATG**AACGGTACAGAGGGACCTATGTTCTACGTGC
CTATGTCCAATGCCACCGGCATTGTCAAGAGCCCATACGACTATCCCCAGTACTACCT
GGTGGCGCCATGGGCATACGGCTGCCTGGCCGCGTACATGTTCTTCC

<2>

TGCCAGCGTGGCCTGGTATATCTTCACCCACCAGGGAAGCGAATTTGGGCCTGTCTTCA
TGACCGTGCCAGCCTTCTTTGCCAAGAGTGCTGCTGTCTACAACCCATGCATCTACATC
TGCATGAACAAGCAGTTCCGTAACTGCATGATCACCACCCTGTGCTGCGGCAAGAACC
CCTTCGAGGAGGAAGAGGGCGCCTCCACTACTGCATCCAAGACCAAGGCTTCGTCCGT
GTCTTCCAGCTCCGTGTCCCCTGCG**TAA**ACAGTTGTCCGTGACACAGAATAAGCAGTG
ACATGCACTGGGCTTCAACGGCAACCGACGACACAGGGACCACAAAGTGTTCAGCCC
AGGGAAACGAGCAACCACTACCACTTGCAGAAAAAAATGTCTGTCAGTTTTCCTTTTT
GTATTTTCACAAAACCCAATTGGTTCAACCAAAAGACAGTTTTGAGAGAGGACAGACC
ATGTCCCAGTTTCAGTACATCCAGCGAGTCCAGCATAACAGTGCATAAGATTTTTTTGA
TTTTTTCTTCCTAAAATGGAGCAAAAGGAAAAATATCTTAACTCTCTTACTGTTGGACT
CCTTATACTGGCTTTGTTGTGATTGTAGAGGCATGTATTCAAGGCAACGTAACAATAAA
AAGCACTTTGCAAATTAAATTGCTGTTTATGTTTTAATTGAGCCGTGATGTTAATAAAAT
GTCAAAATAGTATTTTAAATTAAATAAAAGTGATTTCTGATGAGAGTTTTAATGTGGTG
TATATATTCCCACCGCTAATGTCTTCGGTAAAGGCTGAGTGCTTTTTAATCTATTCTTTTT
AATCATCATCAATACGACAGAGTTTCANA

Figure 6. DNA sequence of the fragment corresponding to the 5'-end and 3'-end of opsin gene (common carp) obtained from DNA Walking. <1> shows 800bp DNA fragment corresponding to the 5'-end region, and bold letter indicates the start codon. <2> shows 850bp DNA fragment corresponding to the 3'-end region, bold letter indicates the stop codon.

5′-GAGTTTAGGTCCAGCGTCCGTGGGGGGGGACGGTCTCCCTCTTCATCACTATCCTACACA
GCCAGAAGAAACACCACTGAAGGGCTGATCGCAACCGCAAGCCGCAACC

●　　●

ATGAATGGAACGGAGGGACCATATTTTTATGTCCCTATGGTAAATACCACCGGCATTGTC 60
M N G T E G P Y F Y V P M V N T T G I V 20
AGGAGTCCTTATGAATACCCTCAGTACTACCTTGTCAGCCCAGCAGCTTATGCTGCCCTG 120
R S P Y E Y P Q Y Y L V S P A A Y A A L 40
GGTGCCTATATGTTTCTGCTCATCCTTGTTGGCTTTCCTGTCAACTTCCTGACTCTCTAC 180
G A Y M F L L I L V G F P V N F L T L Y 60
GTTACCATCGAAAACAAGAAGCTGCGAACCCCTCTAAACTACATCCTTCTGAACCTTGCG 240
V T I E N K K L R T P L N Y I L L N L A 80
◈
GTGGCTAACCTCTTCATGGTGTTTGGAGGATTCACCACAACGATGTACACCTCTATGCAT 300
V A N L F M V F G G F T T T M Y T S M H 100
▼　　■
GGCTACTTCGTTCTGGGTCGTCTTGGCTGCAATCTCGAAGGATTCTTTGCTACACTTGGA 360
G Y F V L G R L G C N L E G F F A T L G 120
GGTGAAATTGCCCTCTGGTCACTCGTTGTTCTGGCTGTTGAAAGGTGGATGGTTGTCTGC 420
G E I A L W S L V V L A V E R W M V V C 140
AAGCCCATCAGCAACTTCCGCTTTGGAGAAAATCATGCTATCATGGGTTTGGCCTTCACC 480
K P I S N F R F G E N H A I M G L A F T 160
TGGTTTGGAGCCAGTGCTTGCGCTGTACCCCCTCTTGTTGGCTGGTCTCGTTACATCCCT 540
W F G A S A C A V P P L V G W S R Y I P 180
▼
GAGGGCATGCAGTGCTCATGTGGAGTTGACTACTACACACGTGCAGAAGGTTTCAACAAT 600
E G M Q C S C G V D Y Y T R A E G F N N 200
GAATCCTTCGTTATCTACATGTTCGTCTGCCACTTCTGCATTCCACTGATTATTGTGTTT 660
E S F V I Y M F V C H F C I P L I I V F 220
TTTTGCTATGGCCGCCTGCTCTGTGCTGTCAAGGAGGCTGCTGCTGCCCAGCAGGAGTCA 720
F C Y G R L L C A V K E A A A A Q Q E S 240
GAGACCACCCAAAGGGCTGAGAGGGAAGTCACCCGCATGGTTGTGATCATGGTTATCGCT 780
E T T Q R A E R E V T R M V V I M V I A 260

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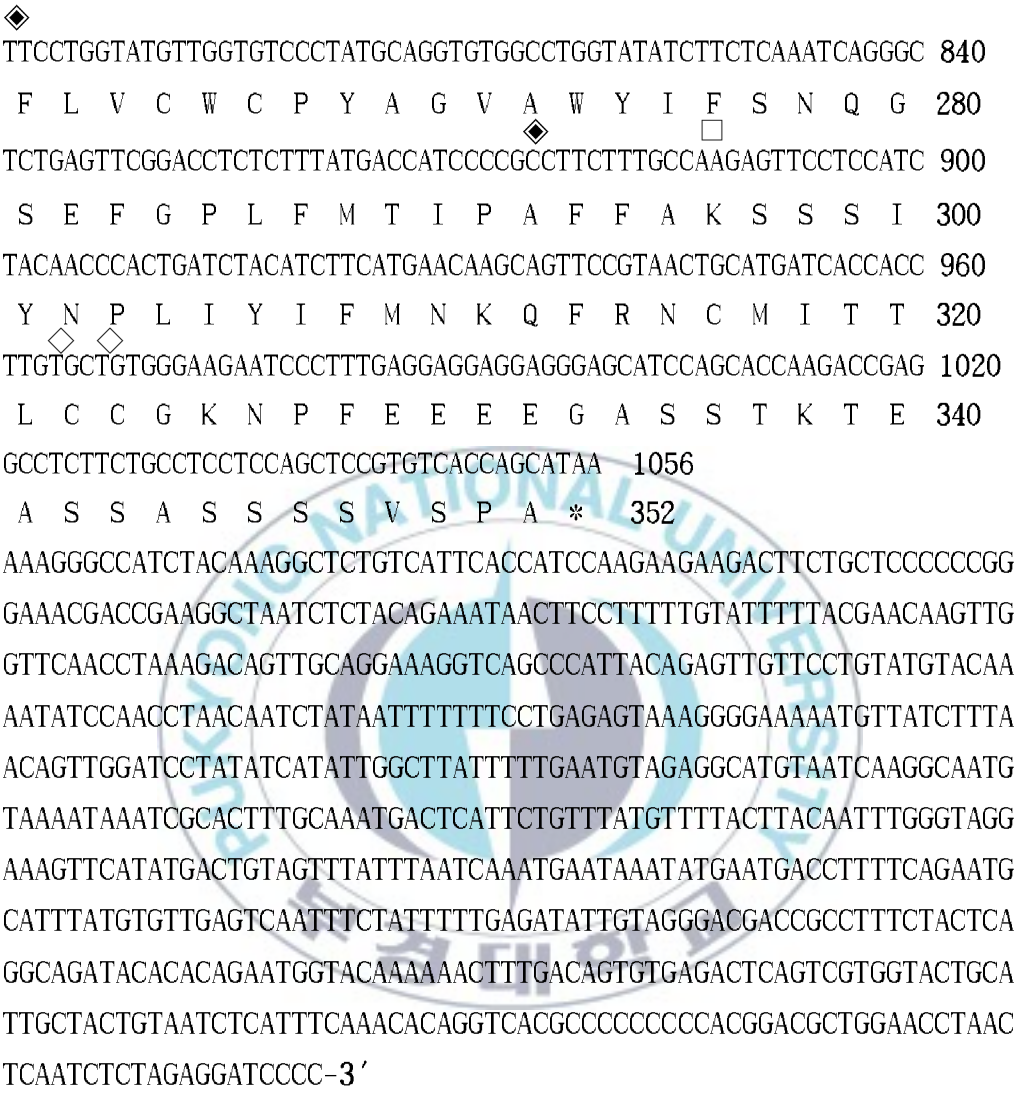

Figure 7. DNA sequence encoding the complete open reading frame of opsin gene and its deduced amino acid sequence of olive flounder (*Paralichthys olivaceus*). The stop codon is indicated by an asterisk (*). Sites involved for Schiff's base formation and its counterion site (□K296 and ■E113), disulfide bond formation (▼C110, C187), glycosylation (●N2, N15) and palmitoylation (◇C322, C323) are indicated. Several amino acid residues implicated in the spectral tuning of rhodopsin are indicated (◈N83, F261, A292)

5′-GAGTTTAGGTCCAGCGTCCGTGGGGGGGGTCGACCTGGTGCAGGATTACCTGGAACAGAG
TGAGAAAGAAAGAGAGAAAAACGGCCCAGAAAGCACTGGGGCTTCCTTATTATAGGGTTTACC
CCCAGGTGCTCTCAATTAGAAAGGGGTGCACCGCACACACTGGCCGACTCCTGTAACTACAAT
AAAAATAAAACAATATTAGAGACAGTCGTCACATCTGTTTCCAGTATCAGTGCATCACTCTGC
TGTTTGAAGTGTAAACACCTCACAGCTAGAC
GAGACAACACTTCTGAAGGACTGATCGAAAAACGCAGCC

●　　●

ATGAACGGCACAGAGGGCCCTAATTTCTACATCCCTATGTCCAATATCACTGGAGTGGTG 60
M N G T E G P N F Y I P M S N I T G V V 20
AGGAGCCCCTTCGAATACCCACAGTACTACCTAGCCGAACCATGGGCCTACACGATCCTG 120
R S P F E Y P Q Y Y L A E P W A Y T I L 40
GCTGCCTACATGTTCACGCTGATTCTCCTGGGCTTCCCCGTCAACTTTCTCACTCTCTAC 180
A A Y M F T L I L L G F P V N F L T L Y 60
GTCACCATCGAGCACAAGAAGCTGAGGACCCCCTTAAATTACATCCTTCTCAACCTGGCT 240
V T I E H K K L R T P L N Y I L L N L A 80

◈

GTGGCCAATCTCTTCATGGTCTTCGGCGGCTTCACCACTACGATGTACACGTCGATGCAT 300
V A N L F M V F G G F T T M Y T S M H 100

▼　　■

GGCTACTTTGTCTTCGGTGAAACAGGCTGCAACCTAGAAGGATACTTTGCTACCCTCGGC 360
G Y F V F G E T G C N L E G Y F A T L G 120
GGTGAAATTTCGCTCTGGTCTCTGGTTGTCCTGGCTATCGAGAGGTGGGTGGTTGTCTGC 420
G E I S L W S L V V L A I E R W V V V C 140
AAGCCAATGAGCAACTTCCGATTTGGTGAGAACCACGCCATCATGGGCTTGGCATTTACC 480
K P M S N F R F G E N H A I M G L A F T 160
TGGATCATGGCCAATACATGTGCTTTGCCTCCTCTGTTTGGATGGTCCAGGTACATCCCA 540
W I M A N T C A L P P L F G W S R Y I P 180

▼

GAAGGCATGCAGTGTTCATGCGGGGTTGACTATTACACCCTCAAGCCTGAAGTCAACAAT 600
E G M Q C S C G V D Y Y T L K P E V N N 200
GAGTCTTTCGTCATCTACATGTTCATAGTTCACTTCTCCATCCCCCTCACCATTATCTCC 660
E S F V I Y M F I V H F S I P L T I I S 220
TTCTGCTACGGCCGACTGGTGTGCACCGTCAAGGAGGCTGCCGCCCAGCAGCAGGAGTCC 720
F C Y G R L V C T V K E A A A Q Q Q E S 240

(continue to next page)

Figure 8. Sequences encoding the complete open reading frame of opsin gene and its deduced amino acid sequence of Japanese eel (*Anguilla japonica*). The stop codon is indicated by an asterisk(*). Sites responsible for Schiff's base formation and its counterion (□K296 and ■E113), disulfide bond formation (▼ C110, C187), glycosylation (●N2, N15) and palmitoylation (◇F322, C323) are indicated. Several amino acids implicated in the spectral tuning of rhodopsin are indicated (◈N83, F261, S292).

5′-ATTTTATTAGCTGTGGCCTAAATAGACTCGTGCTGACAGCCTGGAAACATCAGGTAATCC
CAAGCGAGCCTCTATAAAGCGTGGTGCACGCTCGCCCCGTCAAGTCGTAGCACGGTCCTGCCT
CGTTTCTCCACAGTCCTGCCGAGCCATCCAAACACTACTGCAGAAAGGGGCTGAGCACAACAT
CCAACCGCAGCC

●　　●

ATGAACGGTACAGAGGGACCTATGTTCTACGTGCCTATGTCCAATGCCACCGGCATTGTC 60
M N G T E G P M F Y V P M S N A T G I V 20
AAGAGCCCATACGACTATCCCCAGTACTACCTGGTGGCGCCATGGGCATACGGCTGCCTG 120
K S P Y D Y P Q Y Y L V A P W A Y G C L 40
GCCGCGTACATGTTCTTCCTCATTATCACCGGCTTCCCTATCAACTTCCTCACTCTGTAC 180
A A Y M F F L I I T G F P I N F L T L Y 60
GTCACCATCGAGCACAAGAAGCTGCGTACACCTCTCAACTACATTCTGCTGAACCTCGCC 240
V T I E H K K L R T P L N Y I L L N L A 80
◈
ATTTCTGACCTCTTCATGGTGTTCGGTGGCTTCACCACGACGATGTACACGTCGTTGCAT 300
I S D L F M V F G G F T T M Y T S L H 100
▼　　■
GGCTACTTTGTTTTTGGACGCATTGGCTGCAACCTCGAAGGCTTCTTCGCAACCCTGGGT 360
G Y F V F G R I G C N L E G F F A T L G 120
GGTGAAATGGGCCTTTGGTCCTTGGTGGTGCTGGCCTTCGAGAGGTGGATGGTTGTCTGT 420
G E M G L W S L V V L A F E R W M V V C 140
AAGCCCGTGAGCAACTTCCGCTTCGGAGAGAACCACGCCATCATGGGGGTTGTCTTCACC 480
K P V S N F R F G E N H A I M G V V F T 160
TGGTTCATGGCCTGCACCTGCGCCGTGCCTCCCCTGGTCGGCTGGTCCCGTTACATCCCC 540
W F M A C T C A V P P L V G W S R Y I P 180
▼
GAGGGCATGCAGTGCTCGTGCGGAGTCGACTATTACACTCGCGCCCCTGGCTACAACAAT 600
E G M Q C S C G V D Y Y T R A P G Y N N 200
GAGTCCTTTGTCATCTACATGTTCCTTGTCCACTTCATTATTCCATTAATCGTCATATTC 660
E S F V I Y M F L V H F I I P L I V I F 220
TTCTGCTACGGCCGTCTCGTCTGCACCGTCAAAGATGCCGCTGCCCAGCAGCAGGAGTCT 720
F C Y G R L V C T V K D A A A Q Q Q E S 240

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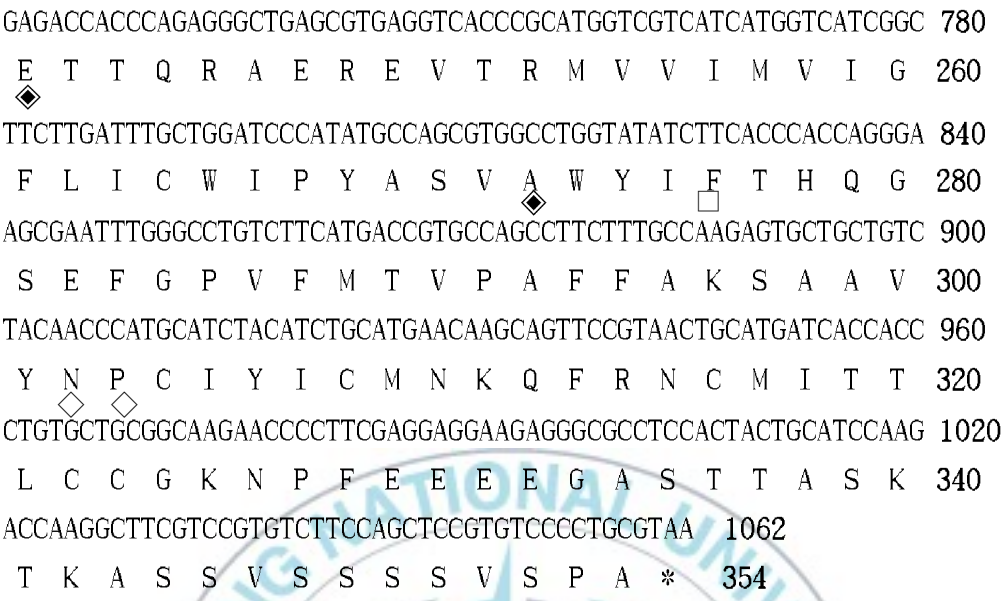

Figure 9. DNA sequence encoding the complete open reading frame of opsin gene and its deduced amino acid sequence of common carp (*Cyprinus carpio*). The stop codon is indicated by an asterisk (*). Sites responsible for Schiff's base formation and its counterion (□K296 and ■E113), disulfide bond formation (▼C110, C187), glycosylation (●N2, N15) and palmitoylation (◇C322, C323) are indicated. Several amino acid residues implicated in the spectral tuning of rhodopsin are indicated (◈D83, F261, A292).

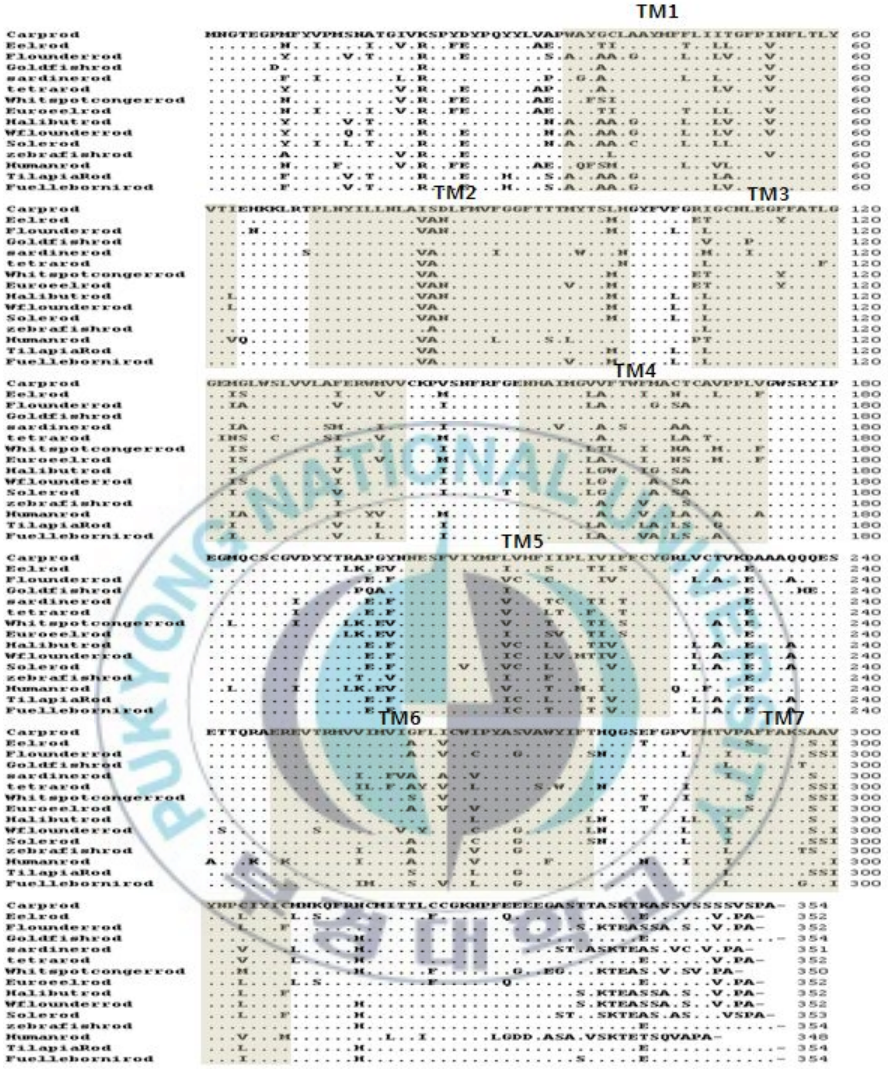

Figure 10. Alignment of rod opsins include Carprod (*Cyprinus carpio*), Flounderrod (*Paralichthys olivaceus*), Eelrod (*Anguill ajaponica*), Goldfishrod (*Carassius auratus*), Sardinerod (*Sardina pilchardus*), tetrarod (*Astyanax mexicanus*), Whitspotcongerrod (*Conger myriaster*), Euroeelrod (*Anguilla anguilla*), Halibutrod (*Hippoglossus hippoglossus*), Wflounderrod (*Pseudopleuronectes americanus*), Solerod (*Solea solea*), Zebrafishrod (*Danio rerio*), Humanrod (*Homo sapiens*), Tilapiarod

(*Oreochromis niloticus*), and Fuellebornirod (*Labeotropheus fuelleborni*). Dots show identical amino acids among species. Shadow boxes indicate region of corresponding to the seven transmembranes.

Table 3. Percent identity rod opsin of olive flounder to those of various species

Species	*Paralichthys olivaceus*	
	Accession number	Rod opsin (%)
Hippoglossus hippoglossus	AF156265	94
Solea solea	Y18672	94
Pseudopleuronectes americanus	AY631036	93
Liza aurata	Y18671	93
Liza saliens	Y18670	93
Mugil cephalus	Y18668	93
Chelon labrosus	Y18669	92
Lithognathus mormyrus	Y18667	91
Dicentrarchus labrax	Y18673	90
Danio rerio	AB087811	85

Table 4. Percent identity between rod opsin of Japanese eels and rod opsins of various species

Species	*Anguilla japonica*	
	Accession number	Rod opsin (%)
Anguilla japonica	AJ249203.1	100
Anguilla anguilla	Q90214	98
Conger conger	O13227	91
Conger myriaster	AB043818	90
Danio rerio	AB087811	85
Rana catesbeiana	P51470	85
Ornithorhynchus anatinus	NM_001127627.1	85
Rana pipiens	P31355	85
Felis catus	NM_001009242.1	84
Macaca mulatta	XM_001094250.1	83

Table 5. Percent identity between rod opsin of common carp and rod opsins of various species

Species	*Cyprinus carpio*	
	Accession number	Rod opsin (%)
Cyprinus carpio	P51488	99
Carassius auratus	P32309	95
Danio rerio	AB087811	92
Oryzias latipes	AB180742.1	86
Poecilia reticulata	DQ912023.1	86
Zosterisessor ophiocephalus	Y18678.1	85
Tetraodon nigroviridis	Q9DGG4	85
Takifugu rubripes	NM_001078631.1	85
Gobius niger	Q9YGZ2	84
Sardina pilchardus	Q9YGZ0	84

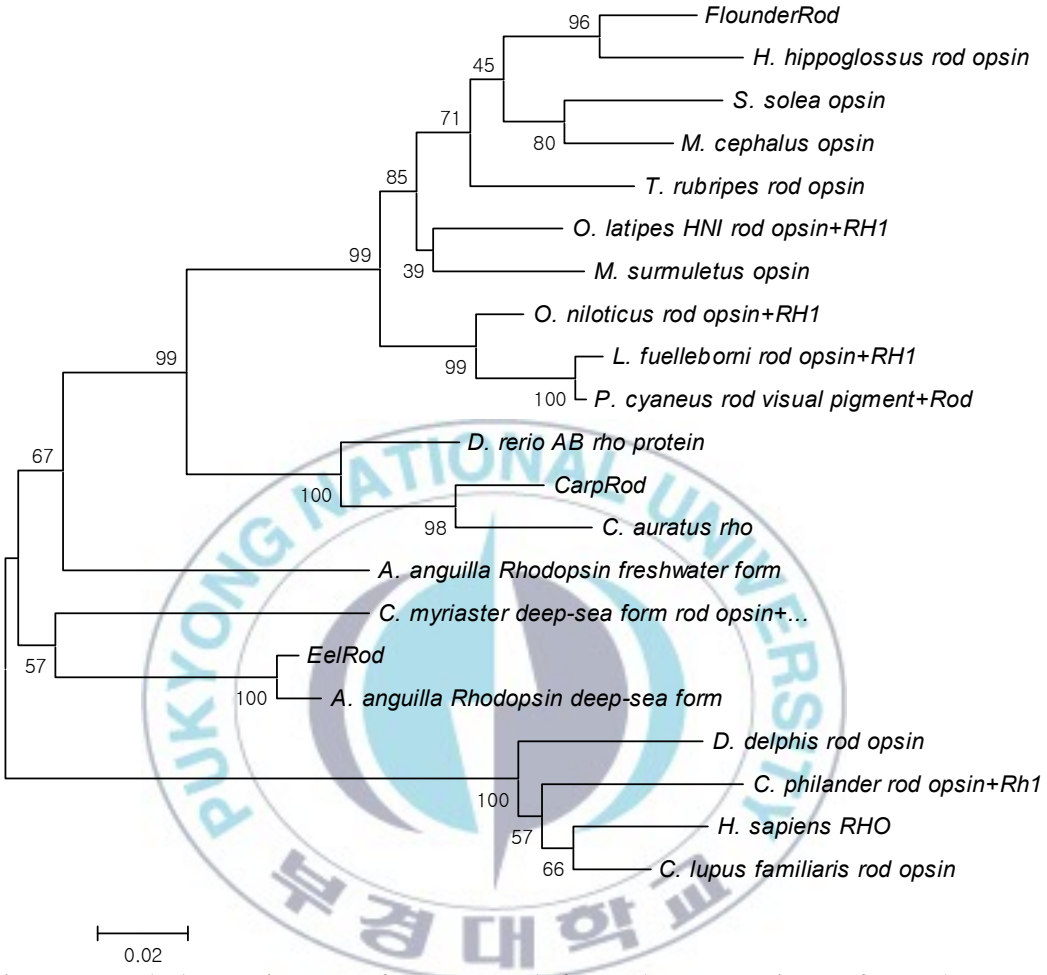

Figure 11. Phylogenetic tree of constructed from the comparison of vertebrate rod opsins by neighbor-joining method using MEGA v 4.0. Node values represent an analysis of 1000 bootstrap trials. Opsins of olive flounder (Flounderrod,*Paralichthys olivaceus*), common carp (Carprod, *Cyprinus carpio*), Japanese eel (*Anguilla japonica*), zebrafish (*Daniorerio,* BC045288.1), Japanese medaka (*Oryzias latipes*, AB180742.1), goldfish (*Carassius auratus,* P32309), European eel (*Anguilla anguilla*, deep water form, Q90214, fresh water form, Q90215), White spotted conger (*Conger myriaster*, fresh water form, BAB21487), common sole (*Solea solea*, CAA77254), Flat head mullet (*Mugil cephalus,*

CAA77250), Atlantic halibut (*Hippoglossus hippoglossus*, AAM17918), Nile tilapia (*Oreochromis niloticus*, AAY26023), *Labeotropheus fuelleborni* (AAY26028), *Paralabidochromis cyaneus* (AAV93304), human (*Homo sapiens*, P08100), Striped red mullet (*Mullussur muletus*, CAA77248), torafugu (*Takifugu rubripes*, AAF44621), Saddle back dolphin (*Delphinus delphis*, AAC12761), Dog (*Canis lupus familiaris*, CAA50502), and bare-tailed wolly opossum (*Caluromys philander*, AAQ82903) were included for the comparison.

Abstract (Korean)

넙치 *Paralichthys olivaceus*, 뱀장어 *Anguilla japonica*, 잉어 *Cyprinus cyprinus* 로부터
Rod opsin 유전자의 클로닝

김 성 완

부경대학교 대학원 수산생물학과

Rhodopsin은 dim-light receptor로써 다양한 세포 외부의 신호를 인지하여 세포 내부로 전달하는 G Protein-coupled receptor (GPCR)을 연구하는데 있어 하나의 모델 시스템으로 이용되어져 왔다. GPCR은 다양한 기능을 수행하는 세포막 단백질 중에서 가장 큰 수용체 집단이다. 어류는 다양한 빛 환경에 서식하고 어류의 시각 시스템은 어류의 서식지에 잘 적응되어져 왔다. 어류에서 spectral tuning mechanism을 연구하기 위해서, 넙치 *Paralichthys olivaceus,* 뱀장어 *Anguilla japonica* 잉어 *Cyprinus carpio*에서 rod opsin 유전자를분리하였다. Genomic DNA 와 PCR을 통해 Rod opsin 유전자를 분리하였으며, 유전자의 염기 서열을 확보하였다. 넙치의 rod opsin 유전자는 1056bp로 이루어져 있으며, 352개 아미노산, 뱀장어와 잉어의 rod opsin 유전자는 두 종 모두 1062bp, 354개 아미노산으로 이루어져 있음을 확인하였다. Rod opsin 아미노산은 rod opsin의 전형적인 특징을 보여 주었는데 Schiff's base formation (K296) 와 counterion (E113), disulfide bond (C110, 187)을 형성하는 두개의 cysteines이 존재하는 것을 확인할 수 있었다. 그러나 뱀장어에서는 palmitoylation site에 cysteine이 아닌, phenylalanine이 존재하는 것을 확인 하였다. 넙치의 rod opsin 유전자는 *Hippoglossus hippoglossus* 와 94%, 뱀장어는 *Anguilla. anguilla* 98%, 잉어는*Cynprius. auratus* 95%의 상동성을 가지는 것을 확인하였다.

Ⅳ. References

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Ⅴ. 감사의 글

지난 2년간 분자생화학 실험실에서 부족한 저를 이끌어 주시고 연구와 실험을 할 수 있게 해 주신 김종명 지도교수님께 진심으로 감사 드립니다. 학부생활 4년과 대학원생활 2년 동안 다양하고 많은 가르침을 주신 손철현 교수님, 허성범 교수님, 장영진 교수님, 김동수 교수님, 조재윤 교수님, 배승철 교수님, 김창훈 교수님 그리고 남윤권 교수님께도 깊은 감사를 드립니다.

또한 많은 시간 실험실에서 함께한 혜정이를 비롯한 실험실 식구들에게도 감사의 마음을 전합니다.

먼저 실험실에서 의대로 전향하고, 함께 제주도로 졸업여행을 떠난 동훈이, 약대 편입 준비 중인 하나, 중국어를 배우기 위해 중국으로 떠난 창연이, 대학원 1년차 때 함께 한 미나, 원이, 프랑스에서 실험하고 있는 보람이, 어려운 임용 시험 준비 중인 선근이, 항상 내 학생증 빌려가 돌려주지 않던 성임이, 지금은 연락 두절된 설희, 크리스마스 카드 써준 은설이, 짜증나는 일이 있을 때 웃으며 작은 힘이 되어준 고은이, 17년간 함께해 온 오랜 친구 정민이, 지금은 수산직 공무원으로 최선을 다하고 있는 호정이, 학부생 시절 즐거운 추억 만들어준 상재, 정완이, 현진이, 실험실은 떠났지만 힘들일 있을 때 애기 나눌 수 있는 경헌형, 그리고 술도 많이 마시고 군대 시절도 함께 한 승수, 제주도에서 아픈 몸보다 선글라스를 더 소중히 한 정호, 정호야 너에게도 주님의 은총이 가득할 꺼다. 시간 날 때 담배 같이 피우던 상엽이, 그리고 마지막으로 실험실에 들어와 고생하고 있는 인철이, 고등학교 시절부터 함께하고, 지금은 아저씨가 된 수휘, 희웅이, 너희 모두와 함께 만들어간 추억들이 너무나 많다. 지난 시간 함께 해 오고, 앞으로도 우리 같이 하자꾸나. 사랑한다.

사랑하는 동생과 항상 저의 곁에서 걱정과 사랑으로 뒷바라지 해 주신 부모님께 너무나 죄송하고 고맙습니다. 대학에서 깨우친 많은 것들을 가슴속 깊이 간직하고, 누군가의 기억에, 가슴에 남는 삶을 살고자 노력하겠습니다.