J. Fish. Sci. Tech., 6(3), 116-124, 2003

JOURNAL OF FISHERIES SCIENCE AND TECHNOLOGY

Molecular Cloning of Seven-band Grouper (*Epinephelus septemfasciatus*) Growth Hormone cDNA and Its Expression in *Escherichia coli*

Jehee Lee*, Helani Munasinghe and Choon Bok Song

Department of Marine Biotechnology, Cheju National University, Jeju 690-756

Isolation and cloning of seven-band grouper (*Epinephelus septemfasciatus*) growth hormone cDNA from pituitary gland revealed an open reading frame of 612 bp coding for a pre-growth hormone of 204 amino acids with a 17 amino acid putative signal peptide. Deduced amino acid sequence showed that there was one possible N-glycosylation site at Asn^{184} and four cysteine residues (Cys^{52}, Cys^{160}, Cys^{177}, Cys^{185}) on the same positions as in some other species where they were involved in the stabilization of the tertiary structure. The seven-band grouper growth hormone (sbgGH) presented a 99.5% amino acid sequence identity with the growth hormone of *Epinephelus coioides* and contained the conserved hormone domain region. Comparison of growth hormone sequences from evolutionarily diverse species revealed 25 amino acid residues conserved in jawless fishes to modern mammals. It also revealed an evolutionary trend to retain the same polypeptide sequence even in the distantly related animals while allowing alterations to occur in polypeptides of the closely related species. In order to create a recombinant system to produce high levels of the growth hormone, it was expressed in *Escherichia coli* (BL21) cells. The gel analysis revealed theoretically expected molecular weights for both mature and pre-sbgGHs.

Key words: *Epinephelus septemfasciatus*, Growth hormone, cDNA, Cloning, Expression

Introduction

Growth hormone is a single chain protein that belongs to a family of proteins including prolactin, placental lactogen and somatolactin with common structural and overlapping biological characteristics (Watahiki et al., 1988). A number of tissues, including brain, pituitary, immune system, placenta, mammary gland and testis are thought to be self-contained units of growth hormone production, regulation and action (Harvey and Hull, 1997). It is essential for the promotion and maintenance of somatic growth and bone maturation.

Growth hormone is involved in a number of important biological activities such as stimulation of linear tissue growth, cellular protein synthesis, intracellular lipolysis, retention of total body potassium and phosphorus and to a lesser extent sodium, and stimulation of synthesis of chondroitin sulfate and collagen as well as the urinary excretion of hydroxyproline. It also shows diabetogenic effect, but the precise mechanism is not known (Hadley, 1996). It is attributed to blocking the action of insulin rather than blocking insulin secretion.

In teleosts, growth hormone is known to be actively involved in osmoregulation. It has already been demonstrated to have several important functions in salmon where it is extensively studied. Growth hormone improves feed conversion during growth, increases appetite, and promotes lipid and glycogen breakdown as well as gluconeogenesis. It is also involved in the seawater adaptation, sexual maturation and it also increases swimming activity as well as dominant feeding behavior and diminishes anti-predator behavior of juvenile salmonids (Bjornsson, 1997).

Human and bovine growth hormones are now produced commercially by genetically engineered organisms for the long-term treatment of children who have growth failure due to an inadequate secretion of normal endogenous growth hormone and for the increment of cows' milk production, respectively. Previous studies show that oral administration of fish growth hormones produced in *Escherichia coli* can improve growth as well as survival rate of fish (Jeh

*Corresponding author: jehee@cheju.ac.kr

et al., 1993; Ben-Aia et al., 1999).

In the present study, the growth hormone cDNA clone from *Perciforme* teleost, seven-band grouper (*Epinephelus septemfasciatus*) was isolated and characterized by sequence analysis and was expressed in *E. coli*, which may be useful in fish culture as a growth enhancer.

Materials and Methods

mRNA extraction and purification

mRNA was extracted directly from *E. septemfasciatus* pituitary gland using Micro-FastTrack™ kit (Invitrogen, Netherland). Cells were lysed in a detergent-based buffer containing RNase/Protein Degrader, incubated at 45℃ and applied directly to oligo(dT) cellulose for the separation of mRNA from DNA, degraded proteins, non-polyadenylated RNAs and cell debris. Quality of RNA was checked in denaturing formaldehyde agarose gel electrophoresis and the quantity was determined spectrophotometrically.

First strand cDNA synthesis

From the mRNA, first strand cDNA was synthesized using the Advantage™ RT-for-PCR Kit (Clontech, USA). The entire population of mRNA molecules is converted into cDNA by priming with oligo(dT)$_{18}$. mRNA was incubated with oligo(dT)$_{18}$ primer at 70℃ for 2 min and quenched on ice. The reverse transcriptase mix containing MMLV reverse transcriptase, recombinant RNase inhibitor, dNTPs and reaction buffer was added to the quenched sample. The mix was incubated at 42℃ for 1 hr for first strand cDNA synthesis. The reaction was stopped by heating at 94℃ for 5 min and diluted with DEPC-treated water.

Amplification of the 3' end of the growth hormone cDNA by RACE PCR.

A primer F1 (5'-GACATGCACAAGGTGGAGAC-3') corresponding to a highly conserved 3' region in fish growth hormones, identified by aligning fish growth hormone sequences, was synthesized. This was used with the oligo(dT)$_{18}$ primer to amplify the 3' end of the growth hormone cDNA using first strand cDNA as the template. The reaction mix contained 1x cloned *Pfu* buffer, 0.2 mM each dNTP, 100 ng first strand cDNA, 250 ng of each primer and 5.0 units of cloned *Pfu* DNA polymerase (Stratagene, USA). The cycling parameters used were denaturation at 94℃ for 45 sec, primer annealing at 53℃ for 45 sec and primer extension at 72℃ for 2 min (25 cycles) with an initial denaturation at 95℃ for 1 min and final extension at 72℃ for 10 min (DNA minicycler PTC-150, MJ research). The PCR product was gel purified, cloned and sequenced.

Amplification of the 5' end of the growth hormone cDNA by RACE PCR.

A poly(G) tail was added to the 3' end of the first strand cDNA synthesized using 2.5 units of terminal deoxynucleotidyl transferase (Amersham Pharmacia Biotech, USA), 1x TdT reaction buffer, 0.25 mM dGTP and 100 ng first strand cDNA in a 100 μL reaction volume at 37℃ for 1 hr. An R1 (5'-GTCTCCACCTTGTGCATGTC-3') primer synthesized corresponding to a conserved region at 3' end in fish growth hormones with an oligo(dC)$_{18}$ primer was used to amplify the 5' end of the growth hormone cDNA using the same conditions used to amplify the 3' end. The PCR product was gel purified, cloned and sequenced.

Amplification of the coding region of growth hormone

The coding region, including the signal peptide, was amplified using F2 (5'-GAGACATATGGACCGAGTCGTCCTC-3') forward primer and the R2 (5'-GAGAGGATCCCTACAGGGTACAGTTGGCCT-3') reverse primer using first strand cDNA as the template and the same conditions used to amplify the 5' and 3' ends.

The coding region without the signal peptide was amplified using the same conditions mentioned above but using F3 (5'-GAGACATATGCAGCCAATCACAGACGGC-3') primer instead of F2 primer and was sequenced.

Expression of growth hormone in *E. coli* [BL21 (DE3)] cells

The coding regions amplified with and without the signal peptide were digested with *Nde*I and *Bam*HI, gel purified and used for cloning into pET11a expression vector (Novagen, USA). The vector was digested with *Nde*I and *Bam*HI and dephosphorylated with shrimp alkaline phosphatase prior to cloning. After ligation, transformed BL21 cells according to the manufacturer's instructions and plated on plates containing ampicilline (100 μL/mL). Colonies were screened for insert by direct colony PCR using Novagen's vector-specific primers.

After the target plasmid was established in BL21 (DE3), expression of the growth hormone gene was induced by adding IPTG (isopropyl-β-D-thiogalactopyranoside) to a final concentration of 1 mM into a growing culture and incubating at 30℃ according to the manual. Cells were induced at OD_{600}=0.6 and harvested 3 hr later. 15 μL of the culture suspended in sample buffer was denatured at 90℃ for 5 min and ran on a 12% SDS gel and stained with Coomassie Blue R-250 staining.

In order to determine the solubility of the proteins expressed, cells grown at 18℃ and 30℃ were harvested and lysed with 3 mg/mL lysozyme in 50 mM Tris-HCl buffer (pH 7.5). After three freeze thawing cycles, cells were sonicated and pelleted by centrifuging at 16,000 g for 20 min at 4℃. The supernatant and cell pellet were separated and ran on a gel as above.

Analysis of nucleotide and amino acid sequences

The N-terminal signal peptide of pre-GH was predicted through SignalP World Wide Web server (http://www.cbs.dtu.dk/services/SignalP/), which incorporates a prediction of cleavage sites and a signal peptide/non-signal peptide prediction based on a combination of several artificial neural networks. Prediction of secondary structure was done by nnpredict (http://www.cmpharm.ucsf.edu/nnpredict.html), a program that predicts the secondary structure type for each residue in an amino acid sequence. The basis of the prediction is a two-layer, feed-forward neural network. sbgGH sequence was compared with other known growth hormone sequences using CLUSTAL W Multiple Sequence Alignment Program (version 1.8, 1999) and all the other analysis were done using BLAST (Basic Local Alignment Search Tool) program developed by the National Center for Biotechnology Information (NCBI) to perform similarity searches on all available sequence data.

Results and Discussion

The cloned cDNA sequence of *E. septemfasciatus* growth hormone contains an open reading frame of 612 bp, coding for a polypeptide with a molecular weight of 23028.3 g/mol (theoretical isoelectric point =6.90) having leucine (15.7%) and serine (12.3%) as the major constituents (Fig. 1). The predicted protein has a putative signal peptide of 17 amino acids, which is subsequently cleaved off during posttranslational modifications. No data are available regarding the amino acid sequence of the mature protein. However, the sequence analysis shows the presence of a stretch of 17 highly hydrophobic residues at the N terminus with a high homology to signal peptides of other fish growth hormones with a signal peptide cleavage site (VSS-QP) between 17^{th} and 18^{th} residues (Nielsen et al., 1997a,b) implying that this region probably contains the signal peptide of the pre-growth hormone. The signal peptide appears to be shorter than those of salmonids [rainbow trout (Agellon and Chen, 1986), chum salmon (Sekine et al., 1989), coho salmon (Gonzalez-Villasenor et al., 1988), and atlantic salmon (Lorens et al., 1989), which has 22 amino acid residues], silurids [indian catfish with 22 amino acids (Anathy et al., 2001)] and mammals [26 amino acids in rat (Rohn and Weigent, 1995), human (Roskam and Rougeon, 1979) and bovine growth hormone (Santome et al, 1973)].

The mature polypeptide contains 187 amino acid residues, which is of the same size as those reported for the mature growth hormones of tuna (Kariya et al., 1989), gilthead seabream (Funkenstein et al., 1991) and yellow tail (Watahiki et al., 1988), but shorter by one amino acid residue than that of growth hormones from several fish species [carp (Chao et al., 1989), chum salmon (Sekine et al. 1985), coho salmon (Gonzalez-Villasenor, 1988), rainbow trout (Rentier-Delrue et al., 1989)]. However, it is longer by one amino acid residue than that of red seabream growth hormone (Momota et al., 1988), which lacks the L^{65} amino acid. Since L^{65} lies in the middle of a stretch of hydrophobic amino acids, its loss may have been compensated by neighboring residues and may not have a critical effect on hormonal activity.

Four Cys residues (Cys^{52}, Cys^{160}, Cys^{177}, Cys^{185}) were found in the mature sbgGH at nearly the same positions as those for other fish, chicken (Lamb et al., 1988) and mammalian [rat (Rohn and Weigent, 1995), bovine (Santome et al., 1973), porcine (Kato et al., 1990), goat (Yamano et al., 1988), horse (Ascacio-Martinez and Barrera-Saldana, 1994), human (Roskam and Rougeon, 1979)] growth hormones. The existence of the two disulfide bonds between these Cys residues was demonstrated in several mammalian growth hormones and their presence was found to be important for the structural integrity and biological activity of the hormone (Anathy et al., 2001). One possible N-glycosylation site (Asn-X-Ser or Asn-X-Thr) is present at the predicted amino acid

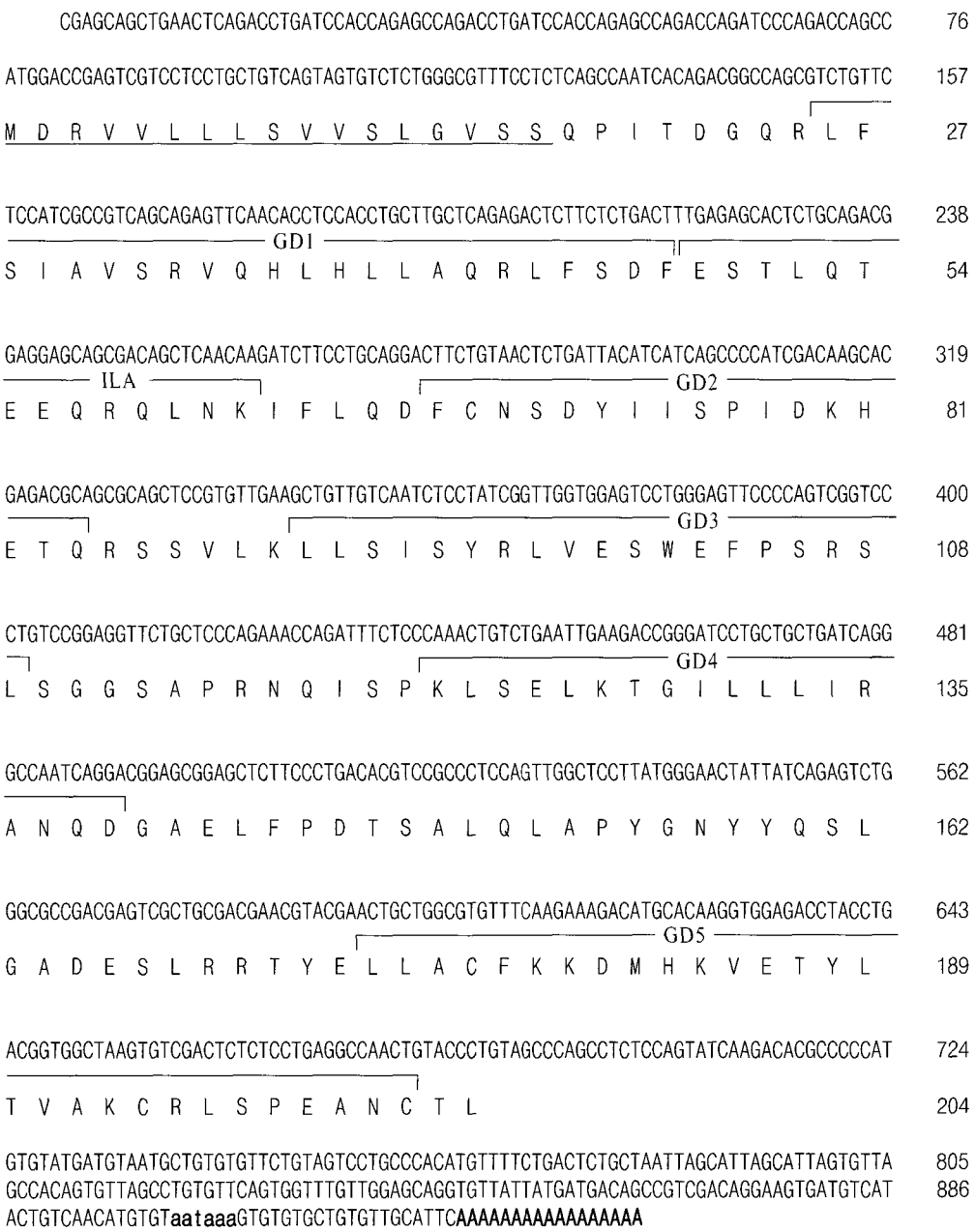

Fig. 1. Nucleotide sequence and the putative amino acid sequence of *Epinephelus septemfasciatus* growth hormone cDNA. The poly(A) tail is highlighted and the polyadenylation signal is given in bold simple case letters. The putative signal peptide sequence is underlined and the evolutionary conserved five domains (GD1 to GD5) and the region correlated with insulin like activity (ILA) are indicated.

sequence of sbgGH at Asn^{184} as in other growth hormones.

According to secondary structure composition predictions, 52.5% of sbgGH is made up of α-helixes and 5.9% of extended strands. It is mainly hydrophilic with a slightly higher hydropathy in the signal peptide region and about 66.2% of residues are expected to expose with more than 16% of their surface to the solvent (http://www.expasy.org/cgi-bin/prostscale.pl). As expected, it contains the conserved hormone domain of somatotropin hormone family, which spans from 5^{th} to 202^{nd} amino acids. The sbgGH also contains a low complexity region spanning from 4-17 amino acids, which is disregarded during comparison

with other sequences using BLAST-P program. The highly conserved region between 148-204 amino acids may be useful in constructing probes to identify growth hormones in other species.

Comparison of sbgGH amino acid sequence with sequences in the "All non-redundant GenBank CDS database" using BLAST-P program and analysis of 450 proteins revealed its maximum homology with the growth hormones of other Pisces species. As expected, the degree of homology decreased in the order of Pisces, Amphibia, Aves, Reptilia and Mammalia. It showed an unusually high identity (67%) to the epidermal growth factor of *Caranx delicatissimus* (hard-tail jack), which was higher than the identity it shared with some other fish growth hormones such as salmon, carps, catfishes, guppy and some eels. Using the hard-tail jack epidermal growth factor sequence as the query, this was rechecked and found to be true. Another interesting finding was the presence of growth hormones in the distantly related taxonomic groups with high homology to sbgGH, compared to the growth hormones from the closely related groups. For example Scorpaeniformes bony fish (*Cottus kazika*) growth hormone, had an 87% identity with *E. septemfasciatus* growth hormone, while the Perciformes bony fish (*Periophthalmus modestus*) sharing only 64% identity. In almost all the taxonomic groups analyzed, the growth hormones with the lower scores in more closely related taxonomic group showed a lower homology to sbgGH sequence than the growth hormones with higher scores in the next distantly related taxonomic level. For example, the bony fish (*Abramis brama*) growth hormone, had a lower sequence similarity to sbgGH than the amphibian (*Xenopus laevis*) growth hormone. This shows an evolutionary trend to retain the same polypeptide sequence even in the distantly related animals while allowing alterations to occur in polypeptides of related species. This complicates the prediction of evolutionary relationships in animals by polypeptide sequence analysis.

```
E. akaara          MDRVVLLLSVVSLGVSSQPITDGQRLFSIAVSRVQHLHLLAQRLFSDFESSLQTEEQRQL
E. awoara          MDRVVLLLSVVSLGVSSQPITDGQRLFSIAVSRVQHLHLLAQRLFSDFESSLQTEEQRQL
E. septemfasciatus MDRVVLLLSVVSLGVSSQPITDGQRLFSIAVSRVQHLHLLAQRLFSDFESTLQTEEQRQL
E. coioides        MDRVVLLLSVVSLGVSSQPITDGQRLFSIAVSRVQHLHLLAQRLFSDFESTLQTEEQRQL
                   **************************************************-*********

E. akaara          NKIFLQDFCNSDYIISPIDKHETQRSSVLKLLSISYRLVESWEFPSRSLSGGSAPRNQIF
E. awoara          NKIFLQDFCNSDYIISPIDKHETQRSSVLKLLSISYRLVESWEFPSRSLSGGSAPRNQIF
E. septemfasciatus NKIFLQDFCNSDYIISPIDKHETQRSSVLKLLSISYRLVESWEFPSRSLSGGSAPRNQIS
E. coioides        NKIFLQDFCNSDYIISPIDKHETQRSSVLKLLSISYRLVESWEFPSRSLSGGSAPRNQIS
                   ************************************************************-

E. akaara          PKLSELKTGILLLIRANQDGAELFPDTSALQLAPYGNYYQSLGADESLRRTYELLACFKK
E. awoara          PKLSELKTGILLLIRANQDGAELFPDSSALQLAPYGNYYQSLGADESLRRTYELLACFKK
E. septemfasciatus PKLSELKTGILLLIRANQDGAELFPDTSALQLAPYGNYYQSLGADESLRRTYELLACFKK
E. coioides        PKLSELKTGILLLIRANQDGAELFPDSSALQLAPYGNYYQSLGADESLRRTYELLACFKK
                   ************************-***********************************

E. akaara          DMHKVETYLTVAKCRLSPEANCTL
E. awoara          DMHKVETYLTVAKCRLSPEANCTL
E. septemfasciatus DMHKVETYLTVAKCRLSPEANCTL
E. coioides        DMHKVETYLTVAKCRLSPEANCTL
                   ************************
```

Fig. 2. Multiple sequence alignment of the known *Epinephelus* growth hormone amino acid sequences. Asterisks show identical sequences while polymorphic regions are shown by dashes. *Epinephelus septemfasciatus* growth hormone had score % of 99.5, 99.0 and 98.5 with *Epinephelus coioides* (AY038606, unpublished), *Epinephelus akaara* (Kang et al., 2003) and *Epinephelus awoara* (AF232711, unpublished), respectively.

```
Gallus          MAPGSWFSPLLIAVVTLGLPQEAAATFPAMPLSNLFANAVLRAQHLHLLAAETYKEFERT 60
Chelonia        ----------------------AFPAMPLSSLFANAVLRAQHLHLLAADTYKEFERT 35
Delphinus       MAAGPRTSMLLAFALLCLPWTQEVGAFPAMPLSSLFANAVLRAQHLHQLAADTYKEFERA 60
Prionace        ------------------------YPLLPLSDLFAKAVHRAQHLHLVAAETTKDFERK 34
Rana            -MASGLGSSLVLLVVICLQSPQGFNAFPRVSLSNLFTNAVIRAQHLHQMVADTYRDYERT 59
Epinephelus     ---------MDRVVLLLSVVSLGVSSQPITDGQRLFSIAVSRVQHLHLLAQRLFSDFEST 51
Petromyzon      MKGGSLAQLLLVMSVLARDAWGRPAARDNDPLRDLNLAIPIAEFVYHLSSQAYAELQEKP 60
                                                *          *          *

Gallus          YIPEDQRYTNKN--SQAAFCYSETIPAPTGKDDAQQKSDMELLRFSLVLIQSWLTPVQYL 118
Chelonia        YIPEEQRHSNKI--SQSASCYSETIPAPTGKDDAEQKSDMELLRFSLILIQSWLNPVQFL 93
Delphinus       YIPEGQRYSIQN--TQAAFCFSETIPAPTGKDEAQQRSDVELLRFSLLLIQSWLGPVQFL 118
Prionace        YIPEEQRHSHKS--SPSAFCQSETIPAPTGKEDAQQRSDRELLLYSLLLIQSWLNPIQNL 92
Rana            YIPEDQRLSNKH--SYSVYCYSETIPAPTDKDNTHQKSDIDLLRFSLTLLQSWMTPIQIV 117
Epinephelus     LQTEEQRQLNKI--FLQDFCNSDYIISPIDKHETQRSSVLKLLSISYRLVESWEFPSRSL 109
Petromyzon      YHGEPPRVAPWSPPNVVMSCHPASWQAPSKKDEVLHKTDDELLRISLEVLESWSG----- 115
                   *  *           *       *  *          **  *     **

Gallus          SKVFTNNLVFGTSDRVFEKLKDLEEGIQALMRELEDR---SPRGPQLLRPTYDKFDIHLR 175
Chelonia        SRVFTNSLVFGTSDRVYEKLRDLEEGIQALMRELEDG---SLRGFQVLRPTYDKFDINLR 150
Delphinus       SRVFTNSLVFGTSDRVYEKLKDLEEGIQALMRELEDG---SPRAGQILKQTYDKFDTNMR 175
Prionace        S-------AFRTSDRVYDKLRDLEEGIFALMKTLEDGG--SSQGFAWLKFSYERFDGNLS 143
Rana            NRVFGNNQVFGNIDRVYDRLRDLDEGLHILIRELDDG---NVRNYGVLTFTYDKFDVNLR 174
Epinephelus     SG------GSAPRNQISPKLSELKTGILLLIRANQDGAELFPDTSALQLAPYGNYYQSLG 163
Petromyzon      ------------VFLRTHAIPLAKQLAAMQRLLQDG-VATMSEGRKPALEFTSVGALPP 161
                                    *          *

Gallus          NEDALLKNY--GLLSCFKKDLHKVETYLKVMKCRRFGESNCTI 216
Chelonia        NEDALLKNY--GLLSCFKKDLHKVETYLKLMKCRRFGESNCTI 191
Delphinus       SDDALLKNY--GLLSCFKKDLHKAETYLRVMKCRRFVESSCAF 216
Prionace        -EEALMKNY--GLLACFKKDMHKVETYLKVMNCKRFAESNCTV 183
Rana            SEEGRAKNY--GLLSCLKKDMHKVETYLKVVKCRRFVESNCTF 215
Epinephelus     ADESLRRTY--ELLACFKKDMHKVETYLTVAKCRLSPEANCTL 204
Petromyzon      VDESLLRNHRQDLLSCFKNDAHRVVTYLKVIKCRRFHDCSKP- 203
                          ** * * * *   ***    *
```

Fig. 3. Global multiple alignment of growth hormone sequences from animals from different lineages. Jawless fish, *Petromyzon marinus* (sea lamprey, BAC15763), vertebrates *Prionace glauca* (blue shark - a cartilaginous fish, P34005), *Epinephelus septemfasciatus* (a bony fish), *Rana catesbeiana* (bullfrog - an amphibian, AAP04356), *Chelonia mydas* (green seaturtle - a reptile, P34005), *Gallus gallus* (chicken - an aves, A60509), *Delphinus delphis* (saddleback dolphin - a mammal, CAD37292) growth hormone sequences were compared and the amino acic residues conserved in all seven lineages are marked with asterisks.

Watahiki et al. (1989) have identified 37 residues, including the four Cysteins, which are conserved in six distinct domains (GD1 to GD5 and ILA) of 20 growth hormones. The regions corresponding to GD1, GD2, GD3 and GD4, which are found on the surface of the molecule, are thought to be involved in specific binding of growth hormone with other residues, while the GD5 domain, which is in the interior of the molecule, is expected to play an important role in structure formation. The presence of three out of the four conserved Cys residues within this GD5 region is an indication of its importance in stabilization of the molecule. The ILA region is believed to be responsible for insulin-like activity. All these 37 residues were found in sbgGH (Fig. 1).

Fish are the oldest vertebrates and are thought to have evolved around 500 million years ago (Powers, 1989) with the Perciformes being the most diversified of all fish orders and the largest vertebrate order (Yowe and Epping, 1995). *E. septemfasciatus*, which belongs to the Perciformes, is the fourth species of *Epinephelus* of which the growth hormone has been sequenced (http://www.ncbi.nlm.nih.gov/).

Amino acid sequence comparisons were carried out for *E. septemfasciatus* growth hormone with the three other known *Epinephelus* growth hormones by

CLUSTAL W Multiple Sequence Alignment Program (version 1.8, 1999) (Fig. 2). These sequence alignments revealed its highest similarity to *E. coioides*, which varied only at the 147th amino acid. Amino acid polymorphisms were seen at 51st, 120th and 147th positions. At 51st position a serine residue found in many bony fishes is replaced by a threonine in *E. septemfasciatus* and *E. coioides*, which lies in the region expected to be responsible for insulin-like activity. This may have been tolerated since both amino acids carry similar functional groups. Other two polymorphisms are found outside the conserved domains.

sbgGH sequence was compared with a number of fish and higher vertebrate hormones belonging to somatotropin hormone family, which will be useful in comparative studies to identify the evolutionary relationships between species and to recognize patterns in evolution of paralogs as well as orthologs, since growth hormone belongs to a family of proteins that is thought to be derived from a common ancestral gene by duplication and divergence, and the ancestral gene, in turn, arose by repeated duplication of a smaller gene or coding domain and insertion of additional domains (Niall et al., 1971; Barta et al., 1981; Miller and Eberhardt, 1983). The global multiple alignment of growth hormone sequences from animals from different lineages (mainly marine animals) unraveled 25 amino acids which are conserved in both ancient vertebrate groups such as Agnatha (jawless fishes), Chondrichthyes (cartilaginous fishes) and modern mammals (Fig. 3). It is interesting to note that ten out of these twenty five residues are restricted to GD5 region which is found inside the molecule. These include three leucines (Leu[174,175,189]), which may probably be involved in the stabilization of the hydrophobic core of the molecule. Threonine[187], tyrosine[188] diad and lysine[179], aspartic acid[181], Histidine[183] charged residues may be involved in the stabilization of the molecule by making hydrophilic interactions among themselves or with trapped water molecules.

Like other teleost growth hormones, sbgGH shows low homology in the ILA region compared with other higher vertebrate groups and *Prionace glauca* (blue shark) growth hormone. This observation may be consistent with the previous studies that teleost growth hormone has very low insulin-like activity (Watahiki et al., 1989).

The sbgGH was expressed in *E. coli* (BL21) cells,

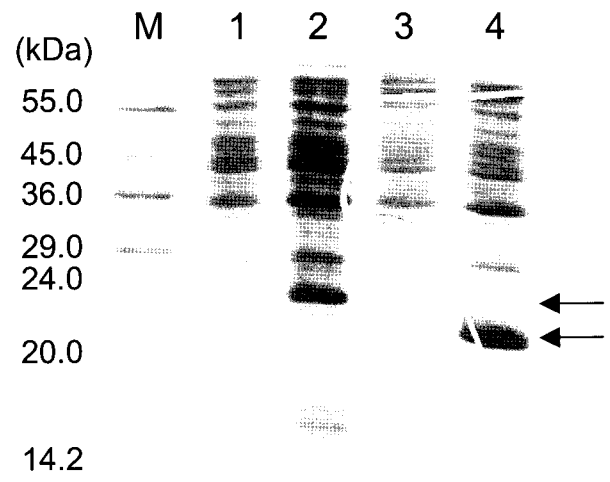

Fig. 4. Analysis of total proteins expressed in *Escherichia coli* (BL21) cells containing *Epinephelus septemfasciatus* growth hormone coding region in a 12% denaturing polyacrylamide gel. Lanes M, SDS-PAGE low range molecular weight standards (Sigma, USA); lanes 1 and 2, *Escherichia coli* (BL21) cells containing *Epinephelus septemfasciatus* pre-growth hormone coding region without and with induction. Lanes 3 and 4, *Escherichia coli* (BL21) cells containing *Epinephelus septemfasciatus* mature-growth hormone coding region without and with induction. The arrows indicate the position of the bands corresponding to pre and mature hormones.

and analysis on a gel resulted in a distinct band with a high intensity in IPTG induced samples compared to the samples without IPTG. The values obtained for the molecular weights of both pre-growth hormone and the mature protein from this were similar to the theoretical values.

Since almost all the sbgGH expressed at 30℃ were in the cell pellet as inclusion bodies, we determined the effect of temperature by expressing it at 18℃. The overall yield decreased with decreasing temperature but resulted in more soluble form, which accounted for 10-20% of the total yield (data not shown). Therefore to obtain the soluble form, it is better to express it at 18℃ than at 30℃. It may also be possible to solubalise the proteins in inclusion bodies using mild detergents such as guanidium hydrochloride and urea. The high level expression of mature growth hormone in *E. coli* suggests the possibility of using this system as a source of sbgGH for aquaculture. We expect to analyse the activity of sbgGH via microinjection into zebrafish embryo.

Acknowlegments

This work was supported by a grant from Ministry of Maritime Affairs and Fisheries. Helani Munasinghe was supported by a research assistant program from the Brain Korea 21 Project.

References

Agellon, L.B. and T.T. Chen. 1986. Rainbow trout growth hormone: molecular cloning of cDNA and expression in *Escherichia coli*. DNA, 5, 463-471.

Anathy, V., T. Venugopal, R. Koteeswaran, T.J. Pandian and S. Mathavan. 2001. Cloning, sequencing and expression of cDNA encoding growth hormone from Indian catfish (*Heteropneustes fossilis*). J. Biosci., 26 (3), 315-324.

Ascacio-Martinez, J.A. and H.A. Barrera-Saldana. 1994. Sequence of a cDNA encoding horse growth hormone. Gene, 43, 299-300.

Barta, A., R.I. Richards, J.D. Baxter and N.L. Eberhardt. 1981. Primary structure and evolution of rat growth hormone gene. Proc. Nat'l. Acad. Sci. USA., 78, 4867-4871.

Ben-Atia, I., M. Fine, A. Tandler, B. Funkenstein, S. Maurice and B. Cavari. 1999. Preparation of recombinant gilthead seabream (*Sparus aurata*) growth hormone and its use for stimulation of larvae growth by oral administration. Gen. Comp. Endocrinol., 113, 155-164.

Bjornsson, B.T. 1997. The biology of salmon growth hormone: from daylight to dominance. Fish Physiol. Biochem., 17, 9-24.

Chao, S.C., F.M. Pan and W.C. Chang. 1989. Purification of carp growth hormone and cloning of the complementary DNA. Biochim. Biophys. Acta, 1007, 233-236.

Funkenstein. B., T.T Chen, D.A. Powers and B. Cavari. 1991. Cloning and sequencing of the gilthead seabream (*Sparus aurata*) growth hormone-encoding cDNA. Gene, 103, 243-247.

Gonzalez-Villasenor, L.I., P.J. Zhang, T.T. Chen and D.A. Powers. 1988. Molecular cloning and sequencing of coho salmon growth hormone cDNA. Gene, 65, 239-246.

Hadley, M. 1996. Endocrinology, 4th ed., Prentice Hall, New Jersey, pp. 89-91.

Harvey, S. and K.L. Hull. 1997. Growth hormone A paracrine growth factor? Endocrine, 7, 267-279.

Jeh, H.S., C.H. Kim, H.K. Lee and K. Han. 1998. Recombinant flounder growth hormone from *Escherichia coli*: overexpression, efficient recovery and growth-promoting effect on juvenile flounder by oral administration. J. Biotechnol., 60, 183-193.

Kang, G.Y., C.B. Song and J. Lee. 2003. Cloning of growth hormone complementary DNA from red-spotted grouper (*Epinephelus akaara*) and its expression in *E. coli*. J. Aquacult., 16, 110-117. (in Korean)

Kariya, Y., N. Sato, L. Kawazoe, S. Kimura, N. Miyazaki, M. Nonaka and H. Kawauchi. 1989. Isolation and characterization of growth hormone from a marine fish, tuna (*Thunnus albacares*). Agric. Biol. Chem., 53, 1679-1687.

Kato, Y., N. Shimokawa, T. Kato, T. Hirai, K. Yoshihama, H. Kawai, M. Hattori, T. Ezashi, Y. Shimogori and K. Wakabayash. 1990. Porcine growth hormone: molecular cloning of cDNA and expression in bacterial and mammalian cells, Biochim. Biophys. Acta, 1048, 290-293.

Lamb, I.C., D.M. Galehouse and D.N. Foster. 1988. Chicken growth hormone cDNA sequence. Nucl. Acids Res., 16, 9339.

Lorens, J., A.H. Nerland, R. Male, I. Lossius, W. Telle and G. Totland. 1989. The nucleotide sequence of Atlantic salmon growth hormone cDNA. Nucl. Acids Res., 17, 2352.

Miller, W.L. and N.L. Eberhardt. 1983. Structure and evolution of the growth hormone gene family. Endocr. Rev., 4, 97-130.

Momota, H., R. Kosugi, H. Hiramatsu, H. Ohgai, A. Hara and H. Ishioka. 1988. Nucleotide sequence of cDNA encoding the pregrowth hormone of red sea bream (*Pagrus major*). Nucleic Acids Res., 16, 3107.

Niall, H.D., M.L. Hogan, R. Sauer, I.Y. Rosenblum and F.C. Greenwood. 1971. Sequences of pituitary and placental lactogenic and growth hormones: Evolution from a primordial peptide by gene reduplication. Proc. Nat'l. Acad. Sci. USA., 68, 866-869.

Nielsen, H., J. Engelbrecht, S. Brunak and G. von Heijne. 1997a. Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Protein Eng., 10, 1-6.

Nielsen, H., J. Engelbrecht, S. Brunak and G. von Heijne. 1997b. A neural network method for identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Int. J. Neural Sys. 8, 581-599.

Powers, D.A. 1989. Fish as model systems. Science, 246, 352-358.

Rentier-Delrue, F., D. Swennen, L. Mercier, M. Lion, O. Benrubi and J.A. Martial. 1989. Molecular cloning and characterization of two forms of trout growth hormone cDNA: expression and secretion of tGH-II by *Escherichia coli*. DNA, 8, 109-117.

Rohn, W.M. and D.A. Weigent. 1995. Cloning and nucleotide sequencing of rat lymphocyte growth hormone cDNA. Neuroimmunomodulation, 2, 108-114.

Roskam, W.G. and F. Rougeon. 1979. Molecular cloning and nucleotide sequence of the human growth hormone structural gene. Nucl. Acids Res., 7, 305-320.

Santome, J.A., J.M. Dellacha, A.C. Paladini, C. Pena, M.J. Biscoglio, S.T. Daurat, E. Poskus and C.E. Wolfenstein. 1973. Primary structure of bovine growth hormone. Eur. J. Biochem., 37, 164-170.

Sekine, S., T. Mizukami, T. Nishi, Y. Kuwana, A. Saito,

M. Sato, S. Itoh and H. Kawauchi, 1985. Cloning and expression of cDNA for salmon growth hormone in *Escherichia coli*. Proc. Nat'l. Acad. Sci. USA., 82, 4306-4310.

Sekine, S., T. Mizukami, A. Saito, H. Kawauchi and S. Itoh. 1989. Isolation and characterization of a novel growth hormone cDNA from chum salmon (*Oncorhynchus keta*). Biochim. Biophys. Acta, 1009, 117-120.

Watahiki, M., M. Yamamoto, M. Yamakawa, M. Tanaka and K. Nakashima. 1989. Conserved and unique amino acid residues in the domains of the growth hormones. J. Biol. Chem., 264, 312-316.

Watahiki, M., M. Tanaka, N. Masuda, M. Yamakawa, Y. Yoneda and K. Nakashima. 1988. cDNA cloning and primary structure of yellow tail (*Seriola quinqueradiata*) pregrowth hormone. Gen. Comp. Endocrinol., 70, 401-406.

Yamano, Y., K. Oyabayashi, M. Okuno, M. Yato, N. Kioka, E. Manabe, H. Hashi, H. Sakai, T. Komano, K. Utsumi and A. Iritani. 1988. Cloning and sequencing of cDNA that encodes goat growth hormone. FEBS Lett., 228, 301-304.

Yowe, D.L. and R.J. Epping. 1995. Cloning of the barramundi growth hormone-encoding gene: a comparative analysis of higher and lower vertebrate GH genes. Gene, 162, 255-259.

(Received June 2003, Accepted September 2003)