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Molecular cloning of Seven-band Grouper growth hormone cDNA and its expression in *Escherichia coli*

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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). In teleosts, growth hormone is known to be actively involved in osmoregulation. Growth hormone improves feed conversion during growth, increases appetite, and promotes lipid and glycogen breakdown as well as gluconeogenesis in salmonids. It is also involved in the seawater adaptation, sexual maturation and it also increases swimming activity as well as dominant feeding behavior and diminishes antipredator behavior of juvenile salmonids (Bjornsson, 1997). Previous studies show that oral administration of fish growth hormones produced in E. coli can improve growth as well as survival rate of fish. In the present study, the growth hormone cDNA clone from 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

First strand cDNA was synthesized from the *E. septemfasciatus* pituitary gland mRNA by priming with oligo(dT)$_{18}$. A primer F1 (5′-GACATGCACAAGGTGGA GAC-3′) corresponding to a highly conserved 3′ region in fish growth hormones was used with the oligo(dT)$_{18}$ primer to amplify the 3′ end of the growth hormone cDNA from the first strand cDNA. A poly(G) tail was added to the 3′ end of the first strand cDNA synthesized and 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. The coding region, including the signal peptide, was amplified

using F2(5'-GAGACATATGGACCGAGTCGTCCTC-3') forward primer and the R2 (5'-GAGAGGATCCCTACAGGGTACAGTTGGC-CT-3') reverse primer using first strand cDNA as the template. The coding region without the signal peptide was amplified in the sameway as above but using F3 (5'-GAGACATATGCAGCCAAT CACAGACGGC-3') primer instead of F2 primer and was sequenced. These were cloned into pET11a expression vector (Novagen, USA) and expressed in BL21 cells at 18℃ and 30℃.

Results

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. The overall protein yield decreased with decreasing temperature but resulted in more soluble form, which accounted for 10-20% of the total yield (data not shown).

References

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