Sinothai Poen 2009: Cloning, Over-expression and Characterization of Growth Hormone from Striped Catfish (*Pangasianodon hypophthalmus*). Master of Science (Genetic Engineering), Major Field: Genetic Engineering, Interdisciplinary Graduate Program. Thesis Advisor: Assistant Professor Somchai Pornbanlualap, Ph.D. 102 pages

Growth hormone (GH) plays a key role in regulation of somatic growth in all vertebrates. In recent years, growth hormone has been utilized in aquaculture industry to enhance growth rate in fish. In this study, the *gh* gene from striped catfish (*Pangasianodon hypophthalmus*) was determined using degenerate primers designed from 5' and 3' untranslated regions through multiple alignments of closely related fish growth hormone cDNA sequences. Similar to other Siluriformes the genomic sequence of the *gh* gene from striped catfish comprised of 5 exons and 4 introns. Intron-exon boundary sequences conformed to the GT-AT rule, which is similar to other GHs. Both the genomic sequence and cDNA sequence of *gh* gene were determined using degenerate primers. The open reading frame of striped catfish *gh* cDNA comprised of 603 base pairs encoded for 200 amino acids, which corresponding to a putative signal peptide of 22 amino acids and the mature protein of 178 amino acids. Subsequently, the striped catfish *gh* cDNA sequence encoding the mature protein was first cloned into pGEM-T easy vector and then transferred to pET-28b expression vector for expressing in *Escherichia coli* strain BL21 (DE3). The recombinant *s*GH was expressed as inclusion bodies upon induction with 1 mM lactose. Eighty four percents of the *s*GH inclusion bodies became soluble when extracted with buffer containing 2 M urea at pH 11. After *in vitro* refolding of the solubilized *s*GH by dialysis and purifying the protein by Ni^{2+}-NTA affinity chromatography, the overall yield of the purified *s*GH was 31.3 mg from 1 liter of cell culture. Far-UV circular dichroism analysis of the *s*GH indicated that it is mostly an alpha helical protein. This result indicated that the protein has been successfully refolded. Since single tryptophan presence in *s*GH, intrinsic fluorescence of *s*GH was monitored for conformational change during unfolding of *s*GH. By fitting fluorescence data into the Boltzmann's equation, a two-state model generated. A value of $[D]_{50\%}$~ 1.72 M urea obtained indicated that when the concentration of urea was 1.72 M, 50% of protein become unfolded. These data indicated that when the concentration of urea is 2 M, the *s*GH did not completely unfolded. When hydrophobic patch of protein is not fully exposed, aggregation of protein decreases during refolding of the sGH. Biological activity of *s*GH was assayed weekly by intra-peritoneal injection for 4 weeks, using the striped catfish and Nile tilapia as tested animals. Growth stimulation was observed in striped catfish injected with 1µg/g of body weight at statistically significant level of 0.05, indicating that recombinant *s*GH had growth stimulating activity on striped catfish. However, no growth stimulation was observed in Nile tilapia. This data suggested that *s*GH could not be recognized by Nile tilapia GH receptor since striped catfish and Nile tilapia growth hormones belong to different orders and share only 52% identity.

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