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Phylogenetic and sequence analysis of the growth hormone gene of two sturgeons, *Huso huso* and *Acipenser Gueldenstaedtii*

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ABSTRACT

In this study, the cDNA Growth Hormone (cGH) of the Belugasturge on (*Huso huso*) and Russian sturgeon (*Acipenser gueldenstaedtii*) were cloned and sequenced, and phylogenetic relationships were examined using nucleic acid and amino acid sequences. The nucleotide sequence of the Beluga GH has an open reading frame of 645 nucleotides encoding a protein 214 amino acid residues. The signal peptide cleavage site was predicted to be at position 72, yeilding a signal peptide of 24 aminoacid residues and a mature peptide of 190 amino acids. The cDNA sequence of the Russian sturgeon was similar to that of the Beluga cGH. The phylogenetic analysis was performed based on amino acid and DNA sequences using the neighbor joining (NJ) and Maximum parsimony (MP) method. Phylogenic trees by the two methods were identical in most of the clades with the high bootstrap support, and the topology of amino acid and DNA sequences showed highest similarity with mammalian sequences.

Key Words: Beluga sturgeon, growth hormone, phylogenetic analysis, Russian sturgeon.

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Introduction

Ancient order Acipenseriforms are the first recognizable believed to date back to the lower Jurassic, approximately 200 million years ago (1).They lived along the coasts of the atlantic and pacific oceans, in the mediterranean and black seas, in rivers, lakes and island seas. The sharp decline of sturgeon populations in their native habitats, mainly in Caspian Sea, is the result of overfishing for meat and caviar, and of the damming of rivers for spawning. These considerations combined with certain life history characteristics make the study of Acipenseridae necessary (2).

Growth Hormone (GH) is a polypeptide hormone, importantfor growth regulation in vertebrates. GH together with prolactin and somatolactin constitute a family of hormones, evolved from a common precursor before the evolution of fish (3). Molecular data from nuclear genes such as the GH gene have been recently used as a source of information in order to evaluate evolutionary relationships of fish at a variety of taxonomic levels, producing (4-7). GH gene is well conserved in evolution, producing phylogenies with substantial statistical confidence.

Due to the importance of GH in fish culture, cDNA encoding GH gene for many teleost fish species have been cloned and sequenced (8-20). But there is very little information on Chondrostean growth hormones (21). The class Chondrostean is ancient and includes fish of high economic value.

In this study, we report the isolation and characterization of GH cDNA of Beluga (*Huso huso*) and Russian sturgeon (*Acipenser gueldenstaedtii*) which belong to the class of Chondrostean fish. Base on the results, a GH based phylogenetic analysis was performed in vertebrates.

Materials and methods

Animals

Three reared beluga supplied by the International sturgeon research Institute in Guilan and two Russian sturgeons caught from zone 2 fisheries of Iran were selected and killed. The pituitary glands were removed and kept in liquid nitrogen.

RNA extraction and cDNA synthesis

Total RNA was extracted from pituitary glands using Biozol solution (Bioflux, Japan). First strand cDNA was synthesized with oligo (dT)18 primers and reverse transcriptase enzyme. All solutions were prepared from DEPC-treated autoclaved distilled water. An upstream primer 5'-ATGGCATCAGGTCTGCTTCT-3' and downstream primer 5'-CTACAGAGTACAGTTGCTCT-3' were designed to synthesize the cDNA encoding of the ORF region of the both sturgeon, corresponding to the submitted sturgeon GH(AY941176.1). PCR was performed in 50 µl reactions using 1 unit *Taq polymerase* (Fermentas, USA) and 35 cycles as follows: 30s of denaturation at 94 ºC, 30s of annealing 64 ºC and 30s of extension at 72 ºC. An initial denaturation for one minute at 94 degrees was performed. A last cycle of 30 min at 72 ºC was also performed. Amplified fragments were eluted from agarose gel and directly ligated to *pTZ57R* and cloned in *E. coli* TOP10 (Invitrogen) using calcium chloride treated competent cells (22). Luria-Bertani (LB) agar plates (1% trypton, 0.5% yeast extract, 1% NaCl, pH=7) containing 50 µg/ml ampicillin were used to screen the recombinant colonies. Plasmids from recombinant colonies were prepared by the alkaline lysis method (Sambrook & Russel, 2001). Insertion of the PCR product was verified by the PCR using T7 promoter

and gene downstream primers, agarose gel electrophoresis and sequencing. Nucleotide and translated amino acid sequences were analyzed using BLASTN and BLASTP (GenBank, NCBI, http://www.ncbi.nlm.nih.gov). The signal peptide and putative cleavage sites were detected using the SignalP (http://www.cbs.dtu.dk/services/SignalP). N glycosylation sites were prognosticated by searching the Asp-Xaa-Ser/Ther motif (http://www.cbs.dtu.dk/services/NetNGlyc). The DNA sequence deposited in the GenBank database(HQ166628.1).

Data analysis

Phylogenetic analysis

Several growth hormone sequences were extracted from NCBI (http://www.ncbi.nlm.nih.gov). Sequences were aligned using Clustalw X (Thompson *et al*, 1997) and phylogenetic analysis was performed using MEGA4 (kimura 2 parameter type) for the construction of the distance matrices, NEIGHBOR (Neighbor-Joining) for the generation of 1000 phylogenetic trees (http://www.megasoftware.net/).

Results

Husohuso growth hormone cDNA sequence

The complete cDNA (645bp) and amino acid sequences of GH of *Huso huso* and *Acipenser gueldenstaedtii* are presented, respectively, in Figures 1 and 2. The cDNA sequences contain an ORF of 645 nucleotides that encode 214 amino acid residues. Based on homology withthe signal peptide of other fish GHs, and also results of the Signal P software, the cleavage site of signal peptide was predicted to be located between amino acids 24 and 25.

The GH hormones exhibit typical GH features such as having four cystein residues capable of forming two disulfide bonds that are assumed to contribute to the tertiary structure of the hormone molecule, a single tryptophan residue, and stretches of amino acids highly conserved in all known GHs. There is only one Asn-Xaa-Thr motif in both GH amino acid sequences at the C terminus region which is a potential site for N- linked glycosylation. The mature form of both GHs contains 190 amino acid residues starting with a tyrosine.

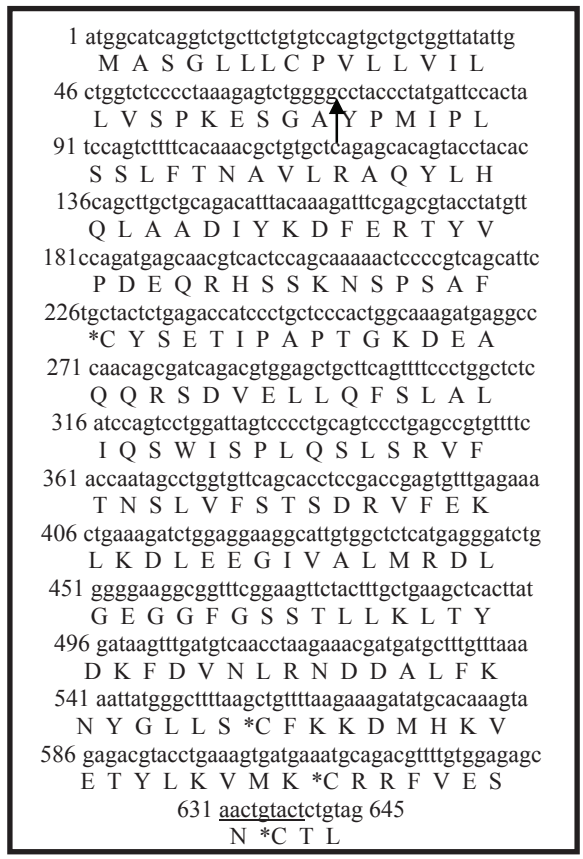


Figure 1. Complete nucleotide sequence of the GH cDNA of sturgeon *Huso huso* and the deduced amino acid sequence of the hormone.

Note. The arrow indicates the probable site for cleavage of signal peptide. The cyctein residues in the mature hormone are show with asteriks. The potential glycosilation site is underlined.

atggcatcaggtctgcttctgtgtccagtgctgctggttatattgctggtctcccctaaa

M A S G L LL C P V L L V I L L V S P K

gagtctggggcctaccctatgattccactatccagtcttttcacaaacgctgtgctcaga

E S G A Y P M I P L S S L F T N A V L R

gcacagtacctacaccagcttgctgcagacatttacaaagatttcgagcgtacctatgtt

A Q Y L H Q L A A D I Y K D F E R T Y V

ccagatgagcaacgtcactccagcaaaaactccccgtcagcattctgctactctgagacc

P D E Q R H S S K N S P S A F C* Y S E T

atccctgctcccactggcaaagatgaggcccaacagcgatcagacgtggagctgcttcag

I P A P T G K D E A Q Q R S D V E L L Q

ttttccctggctctcatccagtcctggattagtcccctgcagtccctgagccgtgttttc

F S L A L I Q S W I S P L Q S L S R V F

accaatagcctggtgttcagcacctccgaccgagtgtttgagaaactgaaagatctggag

T N S L V F S T S D R V F E K L K D L E

gaaggcattgtggctctcatgagggatctgggggaaggcggtttcggaagttctactttg

E G I V A L M R D L G E G G F G S S T L

ctgaagctcacttatgataagtttgatgtcaacctaagaaacgatgatgctttgtttaaa

L K L T Y D K F D V N L R N D D A L F K

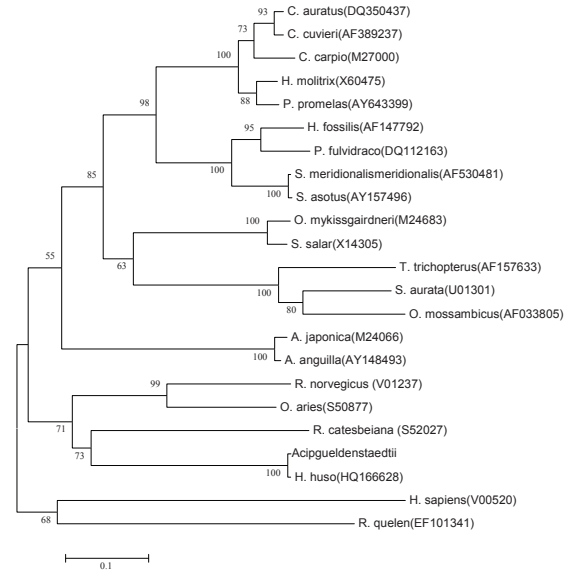
aattatgggcttttaagctgttttaagaaagatatgcacaaagtagagacgtacctgaaa

N Y G L L S C* F K K D M H K V E T Y L K

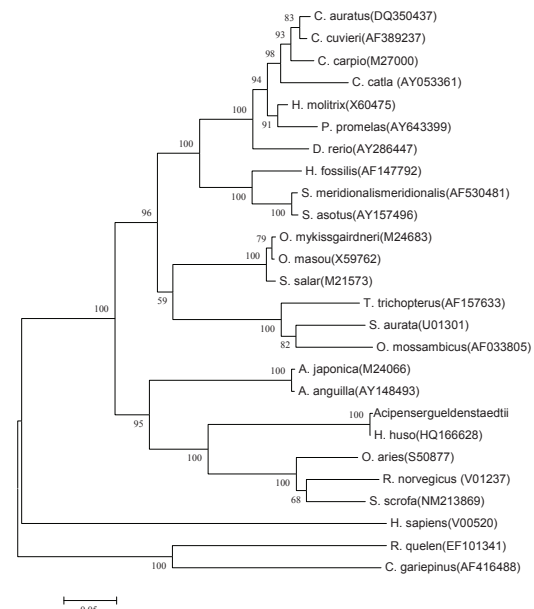
gtgatgaaatgcagacgttttgtggagagcaactatactctgtag

V M K C* R R F V E S N C* T L

Figure 2. Complete nucleotide sequence of sturgeon Acipenser gueldenstaedtii and the deduced amino acid sequence of the hormone. The arrow indicates the possible site for cleavage of signal peptide. The cyctein residues in the mature hormone are asterisked. The potential glycosilation site is undelined.



A



B

Phylogenetic analysis

Results of phylogenetic analysis are presented in Figure 3.

The species whose GH sequences were compared in the analysis and the accession numbers of the sequences used are presented in Table 1.

Figure 3. Phylogenetic trees of selected growth hormone nucleotide sequences A and amino acid residues B. They demonstrate the genetic relationship among vertebrates. The tree was constructed by the neighbor-joining and maximum-parsimony method employing Kimura 2 parameter. Bootstrap consensus values are indicated in the nodes. The use of 1000 replications was chosen for tree construction

Table 1. GH nucleic acid sequences of vertebrate extracted from NCBI and ensemble

No.	Species	Accession no.
1	Danio rerio	AY286447
2	Cyprinus carpio	M27000
3	Spaurus aurata	U01301
4	Tricogaster trichopterus	AF157633
5	Anguilla japonica	M24066
6	Anguilla anguilla	AY148493
7	Hypophthalmichthysmolitrix	X60475
8	Carassius aurata	DQ350437
9	Calta calta	AY053361
10	Clariasgariepinus	AF416488
11	Salmo salar	M21573
12	Onchorinchus keta	X59762
13	Onchorinchus mykiss	M24683
14	Carassius cuvieri	AF389237
15	Heteropneustes fossili	AF147792
16	Paramisgurus dabryanus	DQ350432
17	Rattus norvegicus	V01237
18	Ovisaries	S50877
19	Sus scorfa	NM213869
20	Homo sapiens	V00520
21	Silurus meridionalis	AF530481
22	Silurus asotus	AY157496
23	Rhamdia quelen	EF101341
24	Pimephalespromelas	AY643399

Discussion

This paper describes the molecular cloning, sequence analysis and phylogenetic relationship of sturgeon species *Huso huso* and *Acipenser gueldenstaedtii* GH cDNAs. Despite of a a few deletions and insertions, GH is a significantly conserved protein. The molecule is composed of four conserved regions and four variable regions which are likely to be functionally important. Since the gene GH is a highly conserved protein, it is a good tool for analysis of distantly related species.

The GH nucleic acid and amino acid residues of Beluga and Russian sturgeon had the highest similarity to GH sequences of mammals. These results suggest that the Beluga and Russian sturgeons are primitive fishes that are genetically closer to mammals than to bony fish. The phylogenetic analysis showed that the Beluga and Russian sturgeon GH sequences are more similar to non-fish vertebrate sequences than to sequences of other fish. Like most other species, the GH of the sturgeon species has four cystein residues; 5 cystein residues have been reported in goldfish (19) and in other Cyprinidae (17). These cystein residues participated in formation of 2 disulfide bonds, which have an important role in the biological activity of the hormone (16). There is also a putative glycosylation site in Beluga and Russian sturgeon GH. It has been demonstrated that N linked glycosylation site can serve as a signal for protein transport to the cell surface (23). The Russian sturgeon is a tetraploid (2n=240) and Beluga is a diploid (2n=120) (24-29). Despite the ploidy of the species, only one cDNA sequence type has been cloned. This result implies that there is only one gene copy for GH in these organisms.

The sequences obtained suggest that the Beluga and Russian GH are more similar to the GH of mammals than to that ofother bony fish.

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