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Research Article

Acipenser persicus Growth Hormone Gene Sequencing and its Structures

Ehsan Nasr[1*], Hrachia Hovhannisyan[2], Mohammad Pourkazemi[3]

1- Department of biology, Islamic Azad University of meshkin Shahr, Meshkin Shahr. Iran
2- Scientific and Production Center "Armbiotechnology" NASRA, Armenia
3- Iranian Fisheries Research Organization, P.O. Box: 14155-6116 Tehran, Iran

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Abstract

Administering growth hormone increases growth rate of cultured fish. The aim of this study was isolation and synthesis of Persian sturgeon (GH) cDNA. The total RNA was extracted from pituitary gland of Persian sturgeon and cDNA was synthesized. The full-length cDNA sequence of Persian sturgeon contains a 645 nucleotide open reading frame, which encoding a 214 amino acid protein. The position of the signal peptide cleavage site was predicted to be at position 72. After cleaving of a signal peptide of 24 amino acid residues, a mature peptide of 190 aa formed. The blast observed that the Persian sturgeon pre GH have highest nucleotide sequence similarity with Acipenseridae family and mammals GH. Secondary structure and tertiary structure of Persian sturgeon growth hormone gene were prediction by online software. The secondary structure of the GH revealed the predomination of *a*- helix (> 55%), the domains of high conservation across the vertebrate GH protein. The predicted 3D structure of Persian sturgeon growth hormone attributes to the typical 4-*a*-helix bundle protein conformation, the characteristic 3-D confirmation of growth hormones.

Keywords: Persian Sturgeon, Growth Hormone, cDNA

Introduction

Acipenser persicus is an economically important species for high quality meat and caviar production in the Caspian Sea. It belongs to Acipenseridae family and mainly observed in the Iranian rivers, Sefidrood and Gorgan-chaii, flowing into the Caspian Sea (Khoshkholgh et al., 2011). During the late 20th century Persian sturgeon (PS) population rapidly declined and now it's one of the endangered species of the sturgeon fishes so it is under governmental programming using artificial methods to save the species from extinction (Yousefian, 2011). The maturation of this specie is very long however, males reach maturity at 12 - 14 years and females at 14 - 18 years of age. Due to long and costly rearing of growing conditions such as feeding, control of water temperature and dissolved oxygen reduced sturgeon maturity to 7-9 years in males and 9-12 years in females (Huai Tsai, 1995). For acceleration of fish growth and maturation widely used biotechnological approaches such as optimization of culture condition. The Growth hormone (GH) as a potential growth promoting agent has long been recognized and GH administration has been shown to accelerate growth rate in a number of animals, especially fish such as rainbow trout, Atlantic salmon, Nile tilapia, Coho salmon, among others (Huai Tsai, 1995; Luis FM, 2003; Venugopal, 2002). The growth hormone good conserved among animals and fishes but observed high diversity in molecular weights and amino acid sequences and space specify. Hence, the cloning, characterization and expression of GH genes has been the subject of extensive research during the last decade. However, almost all the described GH sequences are for fishes of Europe and western countries (Venugopal, 2002). In contrast, there is a little information on Acipenseridae family growth hormones amino acid sequence and there are no information about the GH genes or amino acid sequence of *Acipenser persicus*. The aim of this study was the synthesis and sequencing GH cDNA of *Acipenser persicus*.

Materials and Methods

In this study four Persian sturgeons were captured in the southern part of the Caspian Sea and transported to the International sturgeon Research

Corresponding authors E-mail:
* nasr_ehsan1357@yahoo.com

institute (Rasht, Iran). They were killed and immediately manually extracted their pituitary glands and were used for total RNA extraction.

Total RNA was extracted from pituitary glands of all sturgeons using Biozol solution (Bioflux, Japan) and precipitated into 0.5 volum of RNA precipitation solution (1.2 M sodium chloride, 0.8 M disodium citrate and 0.5 vol of isopropyl alcohol. RNA quality was confirmed using a Nanodrop spectrophotometer with absorbance ratios at OD 260/280. The RNA was treated by DNase and reverse transcribed to first strand cDNA using M-MuLV Reverse Transcriptase kit (Fermentas, USA) and oligo dT(18) at 42 °C for 1 h. All solutions were prepared from DEPC treated autoclaved distilled water. The PS preGH cDNA of all fishes were sequenced in Bioneer Co., South Korea and no differences between the male and female's pre GH cDNA sequences were found.

The specific primers used for amplification of cDNA encoding the target genes were designed from alignment of several known acipenseridea GH nucleotide sequences and matches with the first exon region which was retrieved from the NCBI GenBank. [*Acipenser sinensis* (EU119864.1), *Acipenser gueldenstaedtii* (Russian Sturgeon) (AY941176.1) and *Huso Huso* (AB517597.1). The primers were 5'-ATGGCATCAGGTCTGCTT (forward primer) and 5'-CTACAGAGTACAGTTGCTCTC (reverse primer).

The PCR reaction was Primer sets were generated using Primer3 program (http://biotools.umassmed.edu/bioapps/primer3_www.cgi). The PCR was performed under the condition of DNA denaturation at 94ºC (5 min), followed by 35 cycles of denaturation at 94°C (30 sec), annealing at 58°C (90 sec) and extension at 72°C (30 sec), with a final extension at 72°C (25 min). Amplicons were separated by 1.5 % agarose gel electrophoresis and stained with Ethidium bromide.

PCR products of the appropriate size was excised from the gel, purified by an extraction kit (Vivantis, Iran) and sent to Takapozist Company(Iran) for sequencing.

Nucleotide and deduced amino acid sequences were analyzed using BLAST-N and BLAST-P (GenBank, NCBI, http://www.ncbi.nlm.nih.gov). The signal peptide and putative cleavage sites were detected using the Signal-P (http://www.cbs.dtu.dk/services/SignalP). N glycosylation sites were prognosticated by searching the Asp-Xaa-Ser/Thr motif (http://www.cbs.dtu.dk/services/ NetNGlyc). Secondary structure and tertiary structure of PS GH gene were predicted by online software (Guermeur, 1999; Ma J, 2013).

Results

In our study we isolated and synthesized GH cDNA of Persian Sturgeon. The Persian sturgeon preGH cDNA contains an open reading frame of 645 nucleotides starting from ATG codon and ending with TAG stop codon encoding preGH of 214 amino acid residues. The first 24 amino acid residues from the N-terminus are highly hydrophobic (~70% of the amino acids residues of this region are non-polar) and also have a high degree of homology to the signal peptide of other fish GHs, it is assumed that in the Persian sturgeon pre-GH this region probably represent the signal peptide which is cleaved upon hormone secretion. The position of the signal peptide cleavage site was predicted to be at position between 24 and 25 amino acids. After cleaving of the signal peptide formed mature GH containing 190 amino acid residues starting with a glutamic acid (figure 1).

The obtained polypeptide exhibit typical GH feature, such as four Cystein residues, capable of forming two disulfide bonds which are assumed to contribute to the tertiary structure of the hormone, a single tryptophan residue and stretches of amino acid highly conserved in all known GHs. There is only one Asn-Xaa-Thr amino acid motif in GH at the C terminus region which is potential site for N-linked glycosylation. The amino acids Leucine and Serine are dominant, about 25 % of total pool and only one tryptophan is exists.

By means of Sequences Producing Significant Alignments from National Center for Biotechnology Information (NBCI) data base we compared the sequences of PS GH gene with other fish as well as mammalian GH genes sequences, which demonstrated high degree of identity, especially with Acipenseridea's GH sequences, scores denote conserved nucleotides (71–99%) (Table 1).

Thus, the GH coding DNA sequences of Persian sturgeon GH have 99% similarity to Beluga cDNA (*Huso Huso*) GH, 72% to *Sus scrofa* and 73% to mouse. Apart of a few deletions and insertions, GH is a remarkably conserved protein. The molecule is composed of four conserved region and four variable regions which are likely to be functionally important. In contrast to Persian sturgeon and other fish species the GH of goldfish and in other Cyprinidae contain 5 Cystein residues.

Since the gene GH is a highly conserved protein, it provided a better resolution for more distantly related animals. The characteristics some of vertebrates are presented in table 2.

Atg	gca	tca	ggt	ctg	ctt	ctg	tgt	cca	gtg	ctg	ctg	gtt	ata	ttg	ctg	gtc	tcc	cct	aaa
M	A	S	G	L	L	L	C	P	V	L	L	V	I	L	L	V	S	P	K
Gag	tct	ggg	gcc	tac	cct	atg	att	cca	cta	tcc	agt	ctt	ttc	aca	aac	gct	gtg	ctc	aga
E	S	G	A	>Y	P	M	I	P	L	S	S	L	F	T	N	A	V	L	R
Gca	cag	tac	cta	cac	cag	ctt	gct	gca	gac	att	tac	aaa	gat	ttc	gag	cgt	acc	tat	Gtt
A	Q	Y	L	H	Q	L	A	A	D	I	Y	K	D	F	E	R	T	Y	V
cca	gat	gag	caa	cgt	cac	tcc	agc	aaa	aac	tcc	ccg	tca	gca	ttc	tgc	tac	tct	gag	acc
P	D	E	Q	R	H	S	S	K	N	S	P	S	A	F	C*	Y	S	E	T
atc	cct	gct	ccc	act	ggc	aaa	gat	gag	gcc	caa	cag	cga	tca	gac	gtg	gag	ctg	ctt	cag
I	P	A	P	T	G	K	D	E	A	Q	Q	R	S	D	V	E	L	L	Q
ttt	tcc	ctg	gct	ctc	atc	cag	tcc	tgg	att	agt	ccc	ctg	cag	tcc	ctg	agc	cgt	gtt	ttc
F	S	L	A	L	I	Q	S	W	I	S	P	L	Q	S	L	S	R	V	F
acc	aat	agc	ctg	gtg	ttc	agc	acc	tcc	gac	cga	gtg	ttt	gag	aaa	ctg	aaa	gat	ctg	gag
T	N	S	L	V	F	S	T	S	D	R	V	F	E	K	L	K	D	L	E
gaa	ggc	att	gtg	gct	ctc	atg	agg	gat	ctg	ggg	gaa	ggc	ggt	ttc	gga	agt	tct	act	ttg
E	G	I	V	A	L	M	R	D	L	G	E	G	G	F	G	S	S	T	L
ctg	aag	ctc	act	tat	gat	aag	ttt	gat	gtc	aac	cta	aga	aac	gat	gat	gct	ttg	ttt	aaa
L	K	L	T	Y	D	K	F	D	V	N	L	R	N	D	D	A	L	F	K
aat	tat	ggg	ctt	tta	tgc	tgt	ttt	aag	aaa	gat	atg	cac	aaa	gta	gag	acg	tac	ctg	aaa
N	Y	G	L	L	C*	S	F	K	K	D	M	H	K	V	E	T	Y	L	K
gtg	atg	aaa	tgc	aga	cgt	tgt	gtg	gag	agc	aac	tgt	act	ctg	tag					
V	M	K	R	R	F	C*	V	E	S	N	C*	T	L						

Table 1. Sequences producing significant alignments from (NBCI) data base

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Accession	**Family**	**Species**	***Max identity***
JN604534.1	Acipenseridae	*Acipenser persicus*	100%
HQ166628.1		*Huso huso*	99%
AY941176.1		*Acipenser gueldenstaedtii*	98%
KC460212.2		*Acipenser schrenckii*	98%
JX947839.1		*Acipenser baerii*	98%
EU599640.2		*Acipenser sinensis*	96%
EU390781.1	Mammalia	*Sus scrofa*	72%
AF052192.1		*Trichosurus vulpecula*	72%
X02891.1		*Mouse (Mus musculus)*	73%
S50877		*Ovis aries*	71%
V01237		*Rattus norvegicus*	71%
V00520		*Homo sapiens*	74%
EF521480.1	Aves	*Eupodotis ruficrista*	92%
EF521592.1		*Scytalopus magellanicus*	81%
Ay148493	Anguilidea	*Anguilla anguilla*	67%
M24066		*Anguilla japonica*	67%
M24683	Salmonidea	*Onchorhynchus mykiss*	65%
S52027	Amphibia	*Rana catesbiana*	67%
X60475	Cypriniformes	*Carassius auratus*	61%
M27000.1		*Cyprinus carpio*	65%
X60475		*Hypoththalmichtys mulitrix*	68%
AF389237		*Pimephales promelas*	68%

Table 2. The GH characteristics some of vertebrates

Character	PS	Huso huso	Russian
Nucleotides	967	967	980
5' UTR	25	25	39
ORF	645	645	642
Stop codon	TAG	TAG	TAG
Protein	214	214	213
Signal peptid	25 aa	25 aa	24 aa
Mature protoein	190 aa	190 aa	190 aa
Mol. mature Wt (kDa)	~22 kDa	~22 kDa	~ 22kDa
Isoelectric point (pH)	6.31	6.31	6.31
Glycosylation sites	187	187	187
Cystein residues	76, 187, 204 , 212	76, 187, 204 , 212	76, 187, 204 , 212

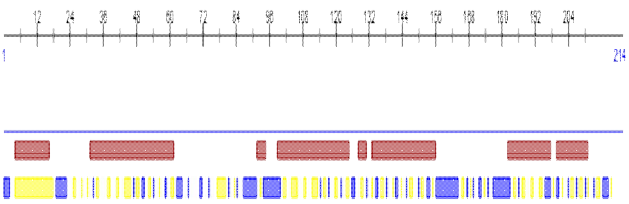
In order to make the PS preGH phylogenetic tree with the GHs of other species, at first PS preGH and several known GHs genes of many species from different species in NBCI were blasted together. The blast revealed that the PS preGH have highest nucleotide sequence similarity to other GH sequences. Thus, Acipenseridae family (98-99%), mammals GH (72%) and Brides GH (*Gallus gallus*) (73%) as well as by Muse (73%), *Trichosurus vulpecula* (72%) (Table 1).

The secondary structure of the GH was determined by in homogenous score combination method of Guermeur *et al.* (1999) based of neural networks (http://npsa-pbil. ibcp.fr/npsa) and by the PROFILESCAN, PEPTIDESORT and other modules of the GCG software (Figure 2).

It is revealed the predomination of *a*- helix (> 55%), the domains of high conservation across the vertebrate GH protein, which attributes to the characteristic 4-*a*-helix bundle confirmation. The four different key *a*-helices are indicated by roman numerals. Second predominant random coils (35%) which connect the helices support the 4 *a*-helix bundle confirmation.

The predicted 3D structure of psGH was made by template-based modeling including alignment and template selection (Ma J, 2013) availeble (http://raptorx.uchicago.edu/download/.) (Figure. 3).

The 56.07% of amino acids are involved in *a*-helix formation, which run anti-parallel to each other. These helices attribute to the typical 4-*a*-helix bundle protein conformation, the characteristic 3-D confirmation of growth hormones.



seq: 1

MASGLLLCPVLLVILLVSPKESGAYPMIPLSSLFTNAVLR
AQYLHQLAADIYKDFERTYV 60
SS:
CCCHHHHHHHHHHHHCCCCCCCCCCCCCCCHHHHHHH
HHHHHHHHHHHHHHHHHHHHHHHHCC
seq: 61
PDEQRHSSKNSPSAFCYSETIPAPTGKDEAQQRSDVELL
QFSLALIQSWISPLQSLSRVF 120
SS:
CCCHHHHHCCCCCCCCCCCCCCCCCCHHHHHHCCHHH
HHHHHHHHHHHHHHHHHHHHHHHHHHH
seq: 121
TNSLVFSTSDRVFEKLKDLEEGIVALMRDLGEGGFGSST
LLKLTYDKFDVNLRNDDALFK 180
SS:
CCCCCCCCCHHHHHHHHHHHHHHHHHHHHHHHCCCCCC
CCCCCCCCCCCCCCCCCCCCHHHHHHH
seq: 181
NYGLLSCFKKDMHKVETYLKVMKCRRFVESNCTL 214
SS:
HHHHHHHHHHHHHHHHHHHHHHHHHHHHECCCCCCCC

Figure 2. The predicted secondary structure of the PS GH protein. Protein sequence is annotated with secondary structure information [a-helix in blue, (H); extended strand in red, (E); random coil, (C)].

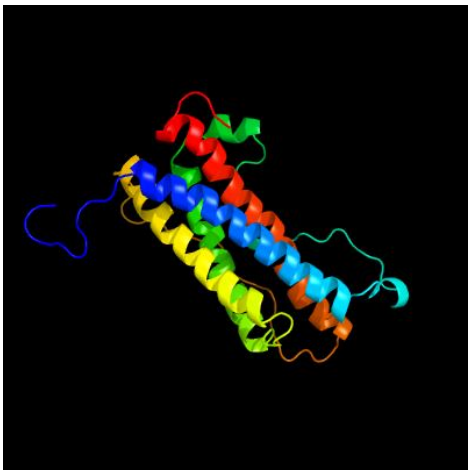


Figure 3. 3-D structure of the mature PS GH.

Discussion

In this study we described sequence analysis and of Persian Sturgeon GH to other vertebrates. Apart from a few deletions and insertions, GH is a remarkably conserved protein. GH molecule is composed of four conserved and four variable regions which are likely to be functionally important. Since the GH gene is a highly conserved

protein, it provided a better resolution for more distantly related species (Luis FM, 2003; Venugopal, 2002).

As a result, the aa sequences of Persian sturgeon (PS) GH have 99% similarity to beluga (Huso Huso) and highest (99%) levels of homology to the GHs of Acipenseridea and mammalian.

The comparison of aa amounts shows no difference between GH of PS and RS and only a little difference between PS and Huso huso. The cystein residues, which are important for the disulfide bond formation and structural integrity of the 3-D structure of the GH protein (Schneider, 1992) is conserved in sturgeons and located at 56, 146,187 and 192 positions. Their presence was found to be important for the structural integrity and biological activity of the hormone. Probably these are the regions, from which strong homology could be drawn between vertebrate GH sequences (Schneider, 1992).

Primary Structure of PS GH Gene contains an ORF nucleotide sequence of the gene (645 bp) and matur sequence (570 bp) with 190 amino acid was determined. The position of signal peptide was in 72 nucleotide (24 amino acid) (fig.1) and this sequence registered in gene bank NCBI for the first time and was given number JN604534.

In the Russian sturgeon (*A. gueldenstaedtii*) growth hormone cDNA nucleotide sequence was 980 bp long and had an open reading frame of 642 bp, beginning with the first ATG codon at position 39 and ending with the stop codon at position 683. The position of the signal peptide cleavage site was predicted to be at position 111, yielding a signal peptide of 24 amino-acids (aa) and a mature peptide of 190 aa. (Yom Din, 2008).

The Beluga sturgeon (*Huso huso*) growth hormone cDNA also 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, yielding a signal peptide of 24 amino acid residues and a mature peptide of 190 amino acids. The cDNA sequence of the Russian sturgeon was similar to that of the Beluga cGH. (Azizzadeh, 2013).

Cao H. et al. (2011) show that the Chinese sturgeon *A. sinensis* GH cDNA consists approximately 954 bp in size including a 16 bp 5′-untranslated region and 296 bp 3′-untranslated region. The open reading frame (642 bp) encodes a 214 aa, but it represents the precursor composed of a 25 aa signal peptide followed by a 189 aa mature polypeptide (Cao, 2011).

Conclusion

These results provide useful information for P.S GH, the finding demonsterate that the GH sequence have a higher degree of identity to mammalian and other fish. These are the first time an *Acipenser persicus* growth hormone encoding cDNA (PSGH; GenBank no. JN604534.1) has been reported and the deduced amino acid sequence obtained. Keeping in view the present results we are here able to suggest future experimental focuses.

Acknowledgment

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