

## Cloning and Sequencing of cDNA that encodes Ostrich Growth Hormone

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### SUMMARY

The cDNA that encodes ostrich (*Struthio Camelus (varietas) domesticus*) growth hormone (oGH) was isolated from an ostrich anterior pituitary cDNA library. The isolated cDNA is 866 base pairs long, containing an oGH coding region of 651 base pairs, which encode a polypeptide of 217 amino acid residues. The amino acid sequence homology between oGH and GHs of ducks, chickens and turkeys was very high (94-90%), while that between oGH and GHs of mice, rats, bovines, goats, and humans was relatively low (72%-71%).

### INTRODUCTION

Growth hormone (GH ; somatotropin) essential for linear growth in vertebrates has been isolated from the pituitaries of several mammalian species [1-12] and lower vertebrates such as chickens [13], turkeys [14], and fish [15, 16]. The primary structure of the GH genes must be compared to study the evolution and regulation of the expression of the genes. GH is produced in the anterior pituitary and secreted into the blood. The secretion of GH is controlled by two neuropeptides produced, which stimulates the secretion of GH from the pituitary, and somatostatin, which suppresses the secretion of GH [17-19]. Some physiological activities of GH are growth stimulation, protein assimilation, carbohydrate metabolism, and lipid metabolism, and electrolyte metabolism. GH stimulates insulin-like growth factor I (IGF-I) transcription in many tissues and IGF-I mediates the function of GH [20]. The ostrich, *Struthio Camelus (varietas) domesticus*, will be an important domesticated animal, but we have no information about its GH gene structure or amino acid sequence.

We cloned a full-length cDNA molecule encoding ostrich GH (oGH), and report here the nucleotide sequence of that cDNA and the deduced primary structure of the precursor protein molecule.

### MATERIALS AND METHODS

*Ostrich pituitary.* A 60 day-old ostrich, *Struthio Camelus (varietas) domesticus* was supplied by Mr. N. Himura, Osaka, Japan. The ostrich was dissected and the anterior pituitary was isolated by Dr. Y. Karasawa (Professor of the Department of Animal Science, Shinshu University). The isolated anterior pituitary was stored

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The nucleotide sequence data reported in this paper have been submitted to the DDBJ/ EMBL/ GenBank nucleotide sequence databases with the accession number AB028191

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in a frozen state at  $-80^{\circ}\text{C}$  until used.

*Polymerase chain reaction (PCR).* For the screening of the 5' terminal region and the 3' terminal region of oGH cDNA, PCR was performed at  $94^{\circ}\text{C}/30\text{ s}$ ,  $55^{\circ}\text{C}/30\text{ s}$ , and  $72^{\circ}\text{C}/60\text{ s}$  for a total of 30 cycles using Ex Taq (Takara Shuzo Co.) according to the supplier's manual. For the screening of full length oGH cDNA, PCR was performed at  $94^{\circ}\text{C}/30\text{ s}$ ,  $58^{\circ}\text{C}/30\text{ s}$ , and  $72^{\circ}\text{C}/40\text{ s}$  for a total of 30 cycles by using LA Taq (Takara Shuzo Co.) according to the supplier's manual.

*PCR primers.* Primer U 1 (22bp: CCT CAG TGG ATG TTG CCT TTA C) and U 2 (20bp: GCC TGT ACG GAA GTG TTA CT) correlate to nucleotide sequences 5091-5112 (forward) and 5119-5138 (forward), respectively, both of which are upstream of the *Bgl*II site (5220), of the cloning vector pAP3neo sequence (GenBank Accession AB003468). Primer L 1 (20bp: CTG GTT CTT TCC GCC TCA GA) and T 3 (20bp: ATT AAC CCT CAC TAA AGG GC) correlate to nucleotide sequences 8-37 (reverse) and 5301-5320 (reverse), respectively, both of which are downstream of the *Not*I site (5295), of the pAP3neo sequence. Primer F 1, F 2, F 3, F 4, R 2, and R 3 are primers corresponding to amino acid sequences of chicken GH which are highly conserved among chicken, duck, and turkey GHs. F 1 (20bp: GCT CAG CAC CTC CAC CTC CT), F 2 (20bp: GCT TCG GTT TTC ACT GGT TC), F 3 (20bp: ATG AGG GAG YTG GAG GAC CG), and F 4 (20bp: GGG AGC TGG GAT GGT TTC TG) corresponded to amino acids 42 to 49 (forward), 102 to 107 (forward), 150 to 156 (forward), and 180 to 186 (forward) of the chicken GH [13], respectively. R 2 (20bp: TCC CTT CTT CYA GGT CCT T) and R 3 (20bp: GGG AGC TGG GAT GGT TTC TG) are primers corresponding to amino acids 139 to 144 (reverse) and 80 to 86 (reverse) of the chicken GH [13], respectively. Forward primer GH1 (25bp: GAG GAA ACG TTC ACT TTC AAG CAA C) was synthesized based on the obtained sequence of 5' terminal region of the oGH cDNA, and reverse primer GH 2 (26bp: CAG CGG GTA GCG GGT TTA TTC TCC TC) was synthesized based on the obtained 3' terminal region of the oGH cDNA.

*DNA sequencing.* DNA sequencing was accomplished using an ABI PRISM Dye Terminator Cycle Sequencing kit (Applied Biosystems) with universal primer U-19 (Takara shuzo Co.) and RV-M (Novagen), and the products were analyzed on a Genetic Analyze System model 373 (Perkin Elmer).

## RESULTS AND DISCUSSION

Total RNA of ostrich anterior pituitary was prepared by the acid guanidium-phenol-chloroform (AGPC) method [21]. Poly(A)RNA was isolated by passing the total RNA solution through a oligotex (dT) 30-super column (Takara Shuzo Co.). First strand synthesis of cDNA was carried out with  $2.5\mu\text{g}$  of the poly (A) mRNA using Superscript Reverse Transcriptase II (Gibco BRL) and RAV-2 reverse transcriptase (Takara Shuzo Co.) with an oligo(dT) 18-linker primer having the *Not*I restriction site. Second strand synthesis was catalyzed using *E. coli* DNA polymerase I in combination with *E. coli* RNase H and *E. coli* DNA ligase. Blunt-end synthesis of the cDNA molecule was done using T4 phage

DNA polymerase. The blunt-end of the cDNA was converted to a terminus that contains a 5' extension by adding a *Bam*HI-*Sma*I adapter (Takara Shuzo Co.). The obtained product was digested with restriction enzyme *Not*I, and ligated with a plasmid vector, pAP3neo, and digested with restriction enzymes *Bgl*II and *Not*I to construct the ostrich anterior pituitary cDNA library.

To screen the 5' terminal region of oGH cDNA, we subjected the ostrich anterior pituitary cDNA library to two rounds of nested PCR with U1, U2, R2 and R3 primers, as summarized in Fig. 1. Forward primers U1 and U2 are vector primers annealing to upstream of the cloning site of pAP3neo, while reverse primers R2 and R3 are primers corresponding to amino acid sequences of chicken GH which are highly conserved among chickens, ducks, and turkeys, as described in MATERIALS AND METHODS. For primary PCR, the diluted ligation mixture for the construction of the ostrich anterior pituitary cDNA library was amplified with the outer primer set, U1 and R2 at concentrations of  $0.2\mu\text{M}$ . An aliquot of the primary PCR reaction product was then reamplified with the inner primer set, U2 and R3. The expected size PCR products were detected as shown in Fig. 2 A. To screen the 3' terminal region of oGH cDNA, we subjected the ostrich anterior pituitary cDNA library to two rounds of nested PCR with L1, T3, F1 and F4 primers, as summarized in Fig. 1. Forward primers F1 and F4 are primers corresponding to amino acid sequences of chicken GH which are highly conserved among chickens, ducks, and turkeys, while reverse primers L1 and T3 are vector primers annealing to downstream of the cloning site of pAP3neo, as described in MATERIALS AND METHODS. For primary PCR, the diluted ligation mixture for construction of the ostrich anterior pituitary cDNA library was amplified with the outer primer set, L1 and F1 at concentrations of  $0.2\mu\text{M}$ . An aliquot of the primary PCR reaction product was then reamplified with the inner primer set, T3 and F4. The expected size PCR products were detected as shown in Fig. 2 B. The amplified DNA fragments removed from the electrophoresis gels were inserted into a PCR product insertion site of pT7BlueT vector (Novagen), and the sequences of the fragments were determined.

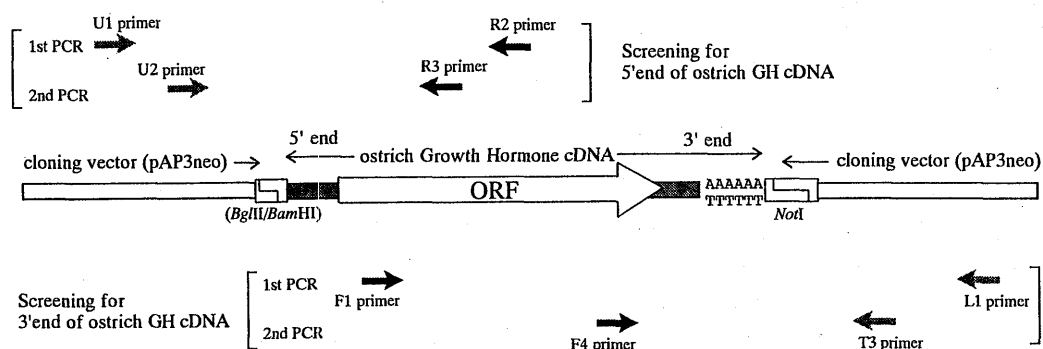


Fig. 1 Schematic representation of primer positions and the nested PCR strategy for screening the 5' terminal and 3' terminal regions of oGH cDNA.

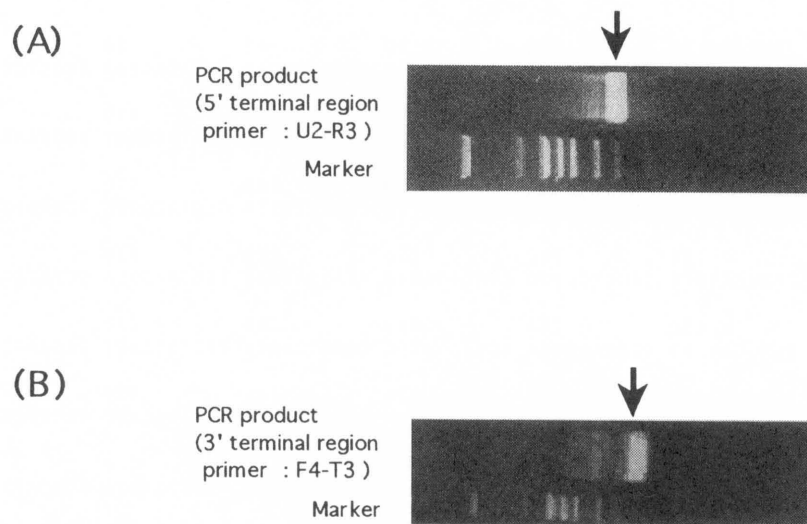


Fig. 2 Gel electrophoresis of nested PCR products. The expected size PCR products from the 5' terminal region of oGH cDNA (A), and those from the 3' terminal region (B), as indicated by the arrows.

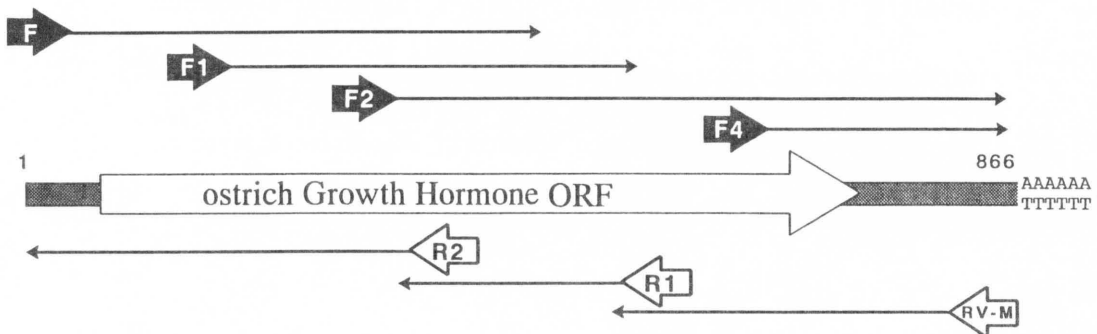


Fig. 3 Schematic representation of primer positions and the sequencing strategy of full length oGH cDNA.

## (A)

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      10      20      30      40      50      60
GAGGAAACGT TCACCTTCAA GCAACACCTG AGCAACCCAT CAGACAGAAA TGGCTCCAGG

      70      80      90     100     110     120
GTCGTGGTTT TCTCCTCTCT TCATTGCTGT GATCACACTG GGATTGCAGT GGCCAAAGGA

      130     140     150     160     170     180
AGCTGCAACC TTCCAGCCA TGCCCTTTC CAACCTGTTT GCCAACGCTG TGCTGAGGGC

      190     200     210     220     230     240
TCAGCATCTT CACCTCCTGG CTGCTGAGAC ATACAAAGAG TTCGAACGCA CCTATATTCC

      250     260     270     280     290     300
GGAGGAACAG AGACACGCCA AAAAAAATTC CCAGTCAGCA TTTTGTACT CAGAAACCAT

      310     320     330     340     350     360
TCCTGCTCCC ACAGGGAAGG ATGATGCCCA GCAGAAATCA GACATGGAGC TTCTTCGGTT

      370     380     390     400     410     420
TTCAGTGGTT CTTATCCAGT CCTGGTTGAC CCCAGTGCAA TACCTAAGCA AGGTGTTTAC

      430     440     450     460     470     480
AAACAATCTA GTTTTGGCA CCTCAGACAG AGTCTACGAA AACTAAAGG ACCTAGAAGA

      490     500     510     520     530     540
AGGGATCCAA GCTCTGATGA GGGAGCTGGA GGATCGGAGC TCCCGCGGGC CGCCGCTCCT

      550     560     570     580     590     600
CAGATCCACC TATGACAAAT TCGACATCCA CCTGCGCAAC GAGGAGGCCC TGCTGAAGAA

      610     620     630     640     650     660
CTACGGCCTG CCGTCCTGCT TCAAGAAGGA CCTGCACAAG GTGGAGACCT ACCTGAAGGT

      670     680     690     700     710     720
GATGAAATGC CGGCGCTTTG GAGAAAGCAA CTGCACCATC TGAGGGAGGA GATGGGCTCC

      730     740     750     760     770     780
TGACCCTGCC GCACGCAGCC CCAGCCCTGG CCCCTCTTCT GCTGAGGAAA AGCAGCCAGG

      790     800     810     820     830     840
GGTCTTTCTC TGTCCTCCC AGGGGTGCCC TCTCGCCTGT AAAGAAAATC GGTTTGGCCC

      850     860     870
GAGGAGAATA AACCCGCTAC CCGCTG...

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## (B)

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      10      20      30      40      50      60
MAPGSWFSPF FIAVITLGLQ WPKEAATFPA MPLSNLFANA VLRAQLHLHL AAETYKEFER

      70      80      90     100     110     120
TYIPEEQRHA NKNSQSAFCY SETIPAPT GK DDAQQKSDME LLRFSVLVLIQ SWLTPVQYLS

      130     140     150     160     170     180
KVFTNNLVFG TSDRVYEKLG DLEEGIQALM RELEDRSSRG PPLLRSYDK FDIHLRNEEA

      190     200     210     220
LLKNYGLPSC FKKDLHKVET YLKVMKRRF GESNCTI...

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Fig. 4 (A) cDNA sequence of oGH cDNA. (B) Deduced amino acid sequence of oGH cDNA

For the PCR screening of the full length oGH cDNA, a forward primer GH 1 and a reverse primer GH 2 were synthesized. GH 1 was synthesized based on the 5'-end 25-bp sequence of the cloned 5' terminal region of oGH cDNA, and GH 2 was synthesized based on the 3'-end 26-bp sequence of the cloned 3' terminal region of the cDNA. The diluted ligation mixture for the construction of the ostrich anterior pituitary cDNA library was amplified with these primers at a concentration of  $0.2 \mu\text{M}$ . The PCR product was subjected to 1.0% agarose gel electrophoresis, and an amplified band corresponding to about 850 bp was detected. DNA was removed from the gel and was cloned to the PCR product insertion site of pT7BlueT vector. The base sequence of the above obtained DNA fragment corresponding to a full length oGH cDNA was determined by using F 1, F 2, R 1, R 2, U-19 primer (Takara shuzo Co.) and RV-M primer (Novagen) (as summarized in Fig. 3).

It was found that the isolated cDNA fragment contains 866 bp (Fig. 4 A). The oGH has a coding region of 651bp, and amino acid synthesis starts from ATG of the 50-52 position and terminates at the termination codon of TGA of the 701-703 position of the isolated cDNA. The deduced amino acid sequence is shown in Fig. 4 B, indicating that oGH consists of 217 amino acid residues. By sequence analysis, the oGH exhibited significant homology with duck, chicken and turkey GHs. The homology scores were 94%, 93%, and 90% at the deduced amino acid level, respectively. In contrast, the similarity scores between oGH and mammalian GH proteins, such as rat (72%), bovine (71%), goat (71%), and human (71%) GHs were not as high as those between oGH and the GHs of birds.

### ACKNOWLEDGEMENT

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## 和文抄録

### ダチョウ成長ホルモンをコードするcDNAのクローン化と塩基配列の決定

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ダチョウ (*Struthio Camelus (varietas) domesticus*) の成長ホルモン (oGH) をコードする cDNA をダチョウ脳下垂体前葉 cDNA より単離した。単離した cDNA は 651 塩基対のコーディング領域を含む 866 塩基対であり、この領域は 216 アミノ酸残基よりなるポリペプチドに相当していた。ダチョウ成長ホルモンのアミノ酸配列は、アヒル、ニワトリ、およびシチメンチョウの成長ホルモンのアミノ酸配列とは非常に高い相同性 (94-90%) を示したが、マウス、ラット、ウシ、ヤギ、およびヒトの成長ホルモンのアミノ酸配列とは比較的低い相同性 (72-71%) しか示さなかった。