Arch. Biol. Sci., Belgrade, 64 (2), 445-449, 2012 DOI:10.2298/ABS1202445D

cDNA CLONING AND SEQUENCING OF OSTRICH GROWTH HORMONE

ABBAS DOOSTI, PAYAM GHASEMI DEHKORDI and HOSEIN KHODABAKHSH

Biotechnology Research Center, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran

Abstract - In recent years, industrial breeding of ostrich (*Struthio camelus*) has been widely developed in Iran. Growth hormone (GH) is a peptide hormone that stimulates growth and cell reproduction in different animals. The aim of this study was to clone and sequence the ostrich growth hormone gene in *E. coli,* done for the first time in Iran. The cDNA that encodes ostrich growth hormone was isolated from total mRNA of the pituitary gland and amplified by RT-PCR using GH specific PCR primers. Then GH cDNA was cloned by T/A cloning technique and the construct was transformed into *E. coli*. Finally, GH cDNA sequence was submitted to the GenBank (Accession number: JN559394). The results of present study showed that GH cDNA was successfully cloned in *E. coli*. Sequencing confirmed that GH cDNA was cloned and that the length of ostrich GH cDNA was 672 bp; BLAST search showed that the sequence of growth hormone cDNA of the ostrich from Iran has 100% homology with other records existing in GenBank.

Key words: *Struthio camelus*, ostrich growth hormone (GH), cloning, sequencing

INTRODUCTION

Struthio camelus are the largest living birds, measuring up to 2.7 m in height and 150 kg in weight. These birds are considered to be seasonal breeders (Deeming, 1999). Male ostriches have jet-black feathers with white wing and tail plumage (big feathers) and bright red or blue skin. The females have grey-brown feathers and skin (Aganga et al., 2003). The ostrich is the only bird that has two toes; other ratites have three or four. Ostriches can live up to 75 years, with 50 years being the average. Adult males can reach 2.7 m in height and weigh as much as 160 kg. Ostriches will set up breeding "attachments," usually pairs or one male and two females. The ostrich will start breeding at about two to three years of age and may continue for up to 20 years (Hassan et al., 2004). Ostriches will start laying eggs around 1 April and continue laying as late as the end of August. Eggs are laid about every other day, with an average of 40 eggs per year. Incubation takes about 42 days (Cooper, 2001, Robinson and Matthee, 1999).

The ostrich is a flightless bird because it does not have the breastbone called a keeled sternum that flying birds have. Their feathers are also different from those of birds that fly: they are fluffy, and do not hook together like flight feathers. They are not waterproof, and in the rain, an ostrich looks very shaggy and drenched (Cooper and Horbanczuk, 2004). Ostrich farming started in the middle of the 19th century in Africa. The ostrich entered Iran for the first time in 1998. Considered as a better climate for ostrich breeding in Iran, changes in growth rate, maturity and time of laying was observed such that the average of reproduction age of ostrich, unlike other areas of the world, was less than two years. Ostriches are omnivores and they eat fruit, seeds, leaves, shoots, and shrubs as well as insects and lizards (Hassan et al., 2004). They get the water they need from the plants they eat.

They also swallow stones to help them digest their food. These birds are an important animal in many livestock industries and, in the developing world, the export of meat and skins is a valuable source of foreign currency (Aganga et al., 2003). The successful growth and reproductive performance of the ostrich depends on good nutrition and the ability of the bird to utilize the mineral and vitamin supplements therein (Cooper and Horbanczuk, 2004). The initial growth of ostriches is controlled by growth hormone (GH) and feeding. Growth hormone is an essential polypeptide required for the normal growth and development of the ostrich. Functions of growth hormone in ostrich are growth stimulation, carbohydrate metabolism, protein assimilation, lipid and electrolyte metabolism. The ostrich could be an important domesticated animal, but information about ostrich gene structure is not available (Komano et al., 1999). The aim of this study was the cloning and sequencing of ostrich growth hormone and comparing it to the results reported by other investigators from other parts of the world.

MATERIALS AND METHODS

Pituitary extract

The ostrich brain was removed rapidly from the neurocranium to expose the pituitary. The pituitary gland was immediately excised and stored at -80°C until used.

RNA extraction and cDNA synthesis

Total RNA was isolated from the pituitary gland tissue using a Qiagen RNA extraction kit (Qiagen, Ltd., Crawley, UK). RNA was reverse transcribed to cDNA with a first strand cDNA synthesis kit (Fermentas, Germany) according to the manufacturer's protocols.

Amplification of ostrich GH cDNA

Ostrich GH cDNA was amplified using *Taq* DNA polymerase (Roche applied science) and the primers specific for the coding region of ostrich GH cDNA. Primers were designed according to the published sequence for growth hormone cDNA of the ostrich (accession number: AB028191). Primer sequences were the following: the forward primer was Ost-GH-F: 5'–TCCTCGAGACAGAAATGGCTCC–3' and the reverse primer was Ost-GH-R: 5'–CCATGGAGATGGTGCAGTTGCTT–3'. Restriction enzyme sites of *Xho*I and *Nco*I (underlined nucleotides in the above sequences) were integrated into the 5' end of primers Ost-GH-F and Ost-GH-R, respectively. In order to amplify ostrich growth hormone cDNA, PCR was performed in a 50 µl total volume containing 1 µg of template cDNA, 1 µM of each primer (Ost-GH-F and Ost-GH-R), 2 mM $MgCl_2$, 200 µM dNTP, 5 µM of 10X PCR buffer and 1 unit of Taq DNA polymerase (Roche applied science). The 30-cycle amplification was performed in a thermal cycler system (Master Cycler Gradiant, Eppendorf, Germany) with the following program: 94°C for 60 s, 58°C for 60 s, 72°C for 60 s. A final 5 min extension was performed at 72°C. The PCR product was analyzed by electrophoresis in 1% agarose gel in 1X TBE buffer and visualized by ethidium bromide staining on UV transilluminator. The agarose gel slice containing the relevant ostrich GH cDNA fragment was excised and purified by gel extraction kit (Bioneer, Koria) according to the manufacturer's recommendation.

Cloning and preparation of ostrich TOPO-GH

Ostrich growth hormone cDNA (as prepared above) was cloned using T/A cloning technique. T/A cloning is one of the most popular methods of cloning the amplified PCR product using Taq and other polymerases. Ostrich GH cDNA fragment ligated to the T-Vector using TOPO T/A cloning kit (pCR8/GW/TOPO, Invitrogen) according to the manufacturer's instructions to obtain TOPO-GH. *XhoI*/*NcoI* restriction analysis, and PCR technique were used to confirm gene cloning. Finally, the recombinant plasmid (TOPO-GH) was sequenced by specific primers and the Sanger sequencing method (Macrogen, Korea) and the sequence of ostrich GH cDNA was submitted to GenBank.

RESULTS

Total RNA was extracted from ostrich pituitary tissue and the cDNA was successfully prepared. PCR amplification with ostrich GH specific primers generated a 672 bp fragment, which was cloned with T/A cloning technique in a T/A vector (pCR 8/GW/TOPO Vector). Chemical competent cells of *E. coli* were transformed with TOPO-GH recombinant plasmid. Plasmid purification and *XhoI/NcoI* restriction endonuclease digestion of TOPO-GH recombinant plasmid, confirmed the correction of GH cDNA cloning. Fig. 1 shows recombinant plasmids after digestion. A 2817 bp large fragment is related to the TOPO vector and the 672 bp fragment is the GH cDNA band. The sequence of the GH cDNA (cDNA of the ostrich in Iran) was submitted successfully to the GenBank (Accession number: JN559394) and compared with GH nucleotide sequences of other records in the GenBank using basic local alignment search tool (BLAST) software; the comparison showed 100% homology with the published GH sequences of ostrich (Accession number: AB028191).

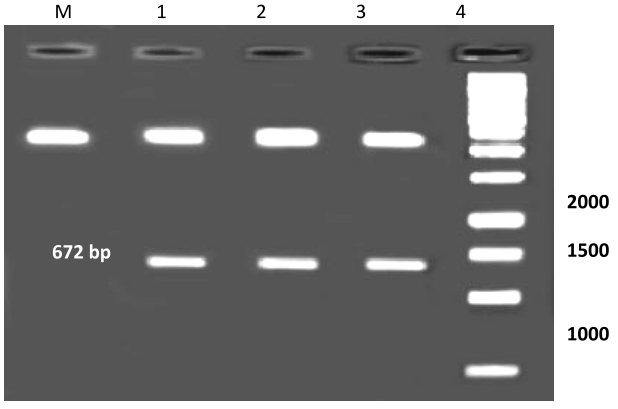

Fig. 1. Analysis of TOPO-GH recombinant vector using *EcoR*I restriction endonuclease enzyme (Line M is 1 kb DNA ladder (Fermentas, Germany), lines 2, 3 and 4 are Topo-GH, and line 4 is Topo vector without GH).

DISCUSSION

The ostrich belongs to the ratite family, i.e. they are flightless birds. This animal, the world's largest bird and one of its oldest (having existed as a species for over 40 million years), is adapted to living in open, arid country (Deeming, 1999). These birds have evolved with a pair of powerful legs capable of propelling it away from danger at speeds up to 70 km per hour (Karklina et al., 2008). Four distinct geographic subspecies are recognized, ranging from the Arabian and Saharan deserts southward throughout Africa. The two subspecies imported to the United States are the Red Neck of Northern Africa and the smaller Blue Neck of Southern Africa (Hassan et al., 2004). The first ostrich farm was founded in 1838 in South Africa, and that country is still the dominant producer of ostriches. The ratite family includes flightless birds with a flat, keelless breastbone (the keel is where the flight muscles are connected). Most of their muscles are in their legs and thighs (Robinson and Matthee, 1999). In the wild, ratites eat seeds, herbaceous plants, insects, and small rodents. Ostriches, rheas and emus are the ratites most commonly raised as livestock in the United States. Ratites produce red meat that is similar to beef or venison, and the hide makes fine leather products. The birds adapt to most climates, so long as they are given proper protection and management (Robinson and Matthee, 1999, Stornelli et al., 2004).

Growth hormone is a polypeptide of fundamental importance for growth regulation in vertebrates and, together with prolactin and somatolactin, constitutes a family of pituitary hormones with similar structure and function that appears to have originated from a common ancestral gene before the evolution of animals (Meier et al., 2006). The long-term effects of GH on somatic growth and regeneration require changes in gene expression that are regulated by sequence-specific transcription factors activated through GH-stimulated signal transduction pathways (Woelfle et al., 2005). As several signaling pathways are stimulated by GH, it is not surprising that a variety of transcription factors serve as mediators of GH action under different conditions. Molecular data and sequencing from nuclear genes such as the GH gene have been recently used as a source of information in order to evaluate the evolutionary rela-

tionships of ostrich at a variety of taxonomic levels, producing phylogenies with substantial statistical confidence (Anathy et al., 2001, Meier et al., 2006).

Considering that the ostriches that exist in Iran are imported from other countries and the short time they have been in this country, the genetic differences should not be significant. In our study, the cDNA of ostrich GH was cloned in the TOPO vector, the recombinant DNA including TOPO-GH was produced and the sequence of the GH cDNA (cDNA of the ostrich in Iran) was submitted to the GenBank. The results of the sequence study of ostrich GH cDNA and its comparison to sequences reported in other records (such as accession number: AB028191 in GenBank), showed 100% similarity.

Recombinant DNA technology is another major DNA-based tool that has gained popular attention in the past decade. This technology allows scientists to find individual genes, cut them out, and insert them into the genome of another organism. Recombinant DNA technology has applications in health and nutrition. In medicine, it is used to create pharmaceutical products such as human insulin (Dyck et al., 2003). In agriculture, it is used to impart favorable characteristics to plants to increase their yield and improve nutritional content. Recombinant DNA technology requires the use of molecular scissors called restriction enzymes, which cut DNA at specific sequences. The cut-out gene is then inserted into a circular piece of bacterial DNA called a plasmid. The plasmid is then re-introduced into a bacterial cell. When the bacteria multiply, the plasmids multiply as well, creating many copies of the gene. Since bacteria multiply very quickly, large numbers of the gene can be produced in the laboratory for further analysis and application (Anathy et al., 2001, Dyck et al., 2003).

Molecular technology is used for the production of many therapeutic proteins, including antibodies, blood products, cytokines, growth factors, hormones, recombinant enzymes and human and veterinary vaccines (Ma et al., 2005). The TOPO-GH plasmid that was generated in this study is ready for sub-cloning and production of recombinant growth hormone protein in future studies.

Acknowledgments - The authors would like to express their gratitude and sincere thanks to the staff of the Biotechnology Research Center of Islamic Azad University of Shahrekord Branch in southwest Iran.

REFERENCES

Aganga, A. A., Aganga, A. O., and *U. J. Omphile* (2003). Ostrich Feeding and Nutrition. *Pakistan J. Nutr*. **2(2)**, 60-67.

Anathy, V., Venugopal, T., Koteeswaran, R., Pandian, T. J., and *S. Mathavan* (2001). Cloning, sequencing and expression of cDNA encoding growth hormone from Indian catfish (*Heteropneustes fossilis*). *J. Biosci*. **26(3)**, 315-324.

Cooper, R. G. (2001). Handling, Incubation, and Hatchability of Ostrich (*Struthio camelus* Var. DOMESTICUS) Eggs: A Review. *J. Appl. Poult. Res*. **10**, 262-273.

Cooper, R. G., and *J. O. Horbanczuk* (2004). Ostrich nutrition: a review from a Zimbabwean Perspective. *Rev. Sci. Tech. Off. Int. Epiz*. **23(3)**, 1033-1042.

Deeming, D. C. (1999). The Ostrich biology, production and health. USA and UK: CAB Publishing, 1-9.

Dyck, M. K., Lacroix, D., Pothier, F., and *M. A. Sirard* (2003). Making recombinant proteins in animals – different systems, different applications. *Trends Biotechnol*. **21(9)**, 394-399.

Hassan, S. M., Siam, A. A., Mady, M. E., and *A. L. Cartwright* (2004). Incubation temperature for ostrich (*Struthio camelus*) eggs. *Poult. Sci*. **83**, 495-499.

Karklina, D., Kivite, J., Dukalska, L., Skudra, L., Sturmovica, E., Kruma, Z., Murniece, I., Galoburda, R., and *M. Sabovics* (2008). Overview of ready to-eat ostrich meat preparation method without decomposition of constituents. *Foodbalt*. 74-78.

Komano, T., Takebe, S., Taguchi, Y., and *H. Sakai* (1999). Cloning and sequencing of cDNA that encode Ostrich growth hormone. Mem. School. B.O.S.T. Kinki University. **5**, 74-81.

Ma, J. K. C., Barros, E., Bock, R., Christou, P., Dale, P. J., Dix, P. J., Fischer, R., Irwin, J., Mahoney, R., Pezzotti, M., Schillberg, S., Sparrow, P., Stoger, E., and *R. M. Twyman* (2005). Molecular farming for new drugs and vaccines. *EMBO Rep*. **6(7)**, 593-599.

Meier, K. M., Castano, C., Laurino, J., Levy, J. A., and *L. F. Marins* (2006). cDNA cloning and phylogenetic analysis of growth hormone genes from the mullet *Mugil platanus* (Mugilo-

morpha, Mugilidae) and the halfbeak *Hemiramphus brasiliensis* (Atherinomorpha, Hemiramphidae). Atlântica, Rio Grande. **28(2)**, 97-102.

Robinson, T. J., and *C. A. Matthee* (1999). Molecular genetic relationships of the extinct Ostrich, *Struthio camelus syriacus*: consequences for Ostrich introductions into Saudi Arabia. *Anim. Cons*. **2**, 165-171.

Stornelli, M. R., Ricciardi, M. P., Giannessi, E., and *A.Coli* (2006). Morphological and histological study of the ostrich (*Struthio camelus* L.) liver and biliary system. *It. J. Anat. Embryol*. **111(1)**, 1-7.

Woelfle, J., Chia, D. J., Massart-Schlesinger, M. B., Moyano, P., and *P. Rotwein* (2005). Molecular physiology, pathology, and regulation of the growth hormone/insulin-like growth factor-I system. *Pediatr. Nephrol*. **20**, 295-302.