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The Bioinformatics analysis of growth hormone or somatotropin structure in animals

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Abstract

For many years, scientists have known. that the crude extract of pituitary glands has substance which effects on growth animals treated with. They named this substance growth hormone. Many years later growth hormone (GH) or Somatotropin (ST) extracted from anterior pituitary glands. Now, it is revealed that ST simulate growth and milk production and reduces the amount of carcass fat in many animals. Studies showed that bovine ST has no effect on human body, it revealed that protein sequences of ST differ in human and animals. In this study bioinformatics analysis of ST protein sequences, using existent data in UniProt database and Gene bank carried out. Results showed that while ST protein sequences is much conserved in animals but, there is many differences among mammals' ST sequences; especially in human, compare to other animals. Based on results of this study, selected animals (*Ovis. aries, Bos. taurus, Capra. hircus, Homo. sapiens, Mus musculus, Rattus norvegicus, Equus caballus, Sus scrofa*) ST arranged into 4 groups, and human ST had most difference with other animals.

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Introduction

About one century ago, Scientists discovered that a crude extract of bovine pituitary stimulated growth of rats (Bauman, 1999). Afterwards, this extract was named as "Growth Hormone" (GH) or "Somatotropin" (ST). The primary function of growth hormone is partitioning of absorbed nutrients and simulating somatic growth in the cell. It also participates in the regulation of nitrogen, lipid, carbohydrate and mineral metabolisms (Revol, 2005). However, it soon revealed that ST did more functions. Russian scientists demonstrated that dairy cow's milk yield was increased by administration of pituitary extract in 1937 (Asimov, 1937). ST has galactopoietics effect which enhanced milk yield in lactating rats and goats (Pinel, 2014). In addition, numerous studies have revealed that ST effectively changes nutrient use in growing animals, and markedly reduces the amount of carcass fat (Bauman, 1998).

Many years after detection of ST, it was isolated from the anterior pituitary in 1945 (LI, 1945). ST is a protein hormone mainly synthesized in and secreted from the anterior pituitary gland of all vertebrates. Currently, it is known that ST is expressed also in extra-pituitary tissues of the immune, nervous, and reproductive systems (Moreno, 2011).

ST is produced by the somatotrop cells which filled 40-50% of anterior pituitary. ST secretion from somatotrop cells is regulated by two well-characterized peptides; Growth hormone-releasing hormone (GHRH, Somatocrinin) and Growth hormone-inhibiting hormone (GHIH, Somatostatin), which released from the hypothalamus (Takahashi *et al.*, 1968). ST is released and inhibited in response to GHRH and GHIH, respectively. During world war II in the 1940s, British scientists performed studies to appraise the potential of using bovine ST (bST) to increase yield of animals and help alleviate food shortages. But, it was not successful because the amount of bST that could be extracted from the pituitary glands of slaughtered animals was not sufficient to be useful in commercial production (Young, 1947). With the progress in biotechnology, recombination of DNA and mass production of recombinant ST became a reality by the early 1980s. human ST or hST, has been used in human medicine to treat growth deficiencies such as dwarfism. In agriculture it is used in pigs and fish farming as a general growth promoter and in dairy cattle to increase milk production (Le Breton and Cesbron, 2008).

ST is released into the bloodstream and subsequently involves two direct and indirect effects on tissues. In the direct path, it binds to growth hormone receptor (GHR) in the target organs such as liver and initiate intracellular signaling pathways and indirect effects that are mostly mediated by insulin-like growth factors, IGF-I and growth related protein kinases (Venugopal, 2002).

The ST gene is comprised of five exons separated by four introns and consists of approximately 654 base pairs (Salama, 2010). The human ST precursor is a single chain polypeptide formed from two parts; signal peptide (first 26 amino acids) and chain (191 length amino acid from 27-217 positions in precursor polypeptide). ST mature protein is that 191 residues chain. While ST gene and protein have highly conserved sequences in animals but, there are many differences along protein and gene of ST between animals, for example, Bovine ST (bST) has no effect on human growth (CARR, 1976). This show that bST and hST structure differs very much. In the present study bioinformatics tools used to analysis ST protein diversity in animals. It provide good information about ST protein structure and differences of ST protein sequence among animals selected for analysis (*Ovis. aries, Bos. taurus, Capra. hircus, Homo. sapiens, Mus musculus, Rattus norvegicus, Equus caballus, Sus scrofa*).

Material and methods

In the current study all protein sequences of ST obtained from Swiss-Prot database, which is manually annotated and reviewed (available at www.uniprot.org). ST *3D structure (PDB file)*

downloaded from the gene bank (available at ncbi.nlm.nih.gov/Structure) and then colored using PyMOL 1.3.

Multiple alignments of ST protein sequences of all selected animals carried out using Clustal omega (available at http://www.ebi.ac.uk/Tools/msa/clustalo/).

Regarding to the evolutionary history was inferred using the Neighbor-Joining method (N. Saitou, 1987), Phylogenetic tree drawn by Mega 6.0 software with the Neighbor-joining method and bootstrap 800 which is based on the number of amino acid substitutions per site.

Results and discussion

All studied animals divided into two groups based on ST protein sequence length. Group one including *Ovis aries*, *Bos taurus*, *Capra hircus* and *Homo sapiens* with 217 AA residues, and group two including *Mus musculus*, *Rattus norvegicus*, *Equus caballus* and *Sus scrofa* which have 216 AA residues. In the animals of group one metal (Zinc) binding site is laid between amino acid 46 and 199; (except Homo. sapiens, 44 and 200), and in group two it lies between amino acid 45 and 198 (Table 1). While two groups different in ST precursor protein but, signal peptide of all animals consist of 26 amino acid residues and so, differences of animals in the protein length related to chain (create mature functional protein).

Table 1. ST protein sequences metal binding and disulfide bond sites, and length of signal peptide and chain in animals.

Name	Amino acid	Metal binding	Disulfide bond		Signal peptide	Chain (Length)
Ovis. aries	217	Zinc 46,199	79↔190	207↔215	1-26	27-217 (191)
Bos. taurus	217	Zinc 46,199	79↔190	207↔215	1-26	27-217 (191)
Capra. hircus	217	Zinc 46,199	79↔190	207↔215	1-26	27-217 (191)
Homo. sapiens	217	Zinc 44,200	79↔191	208↔215	1-26	27-217 (191)
Mus musculus	216	Zinc 45,198	78↔189	206↔214	1-26	27-216 (190)
Rattus norvegicus	216	Zinc 45,198	78↔189	206↔214	1-26	27-216 (190)
Equus caballus	216	Zinc 45,198	78↔189	206↔214	1-26	27-216 (190)
Sus scrofa	216	Zinc 45,198	78↔189	206↔214	1-26	27-216 (190)

With the all animals of two groups Zinc lies between Histidine and Phenylalanine amino acids. There are two disulfide bonds between four cysteine in the ST mature protein; first at the positions 79 and 190 for group one (191 for *Homo sapens*) and amino acid 78-189 for the second group. The second disulfide bond laid at the positions 207-215 for group 1 (*Homo sapiens* 208-215) and positions 78-189 for the second group.

Regarding to that disulfide bond created just between cysteine amino acid, it is distinguishable that one insertion mutation between amino acid 79-191 occurred in *Homo sapiens*; and results of multiple alignment showed that this amino acid is a serine at position 135. Another insertion in *Homo sapiens* ST protein sequence located at amino acid 72 (Leucine) on the multiple alignment result (Fig 2).

It also visible that, 3D Structure of ST (hST) mature folded protein totally has four alpha-helix in its secondary structure which differently colored. Also,

Histidine and Phenylalanine amino acids, which connected to zinc laid exactly adverse to each other, approximately in the middle of folded mature protein on the different alpha helix structures (Fig. 1).

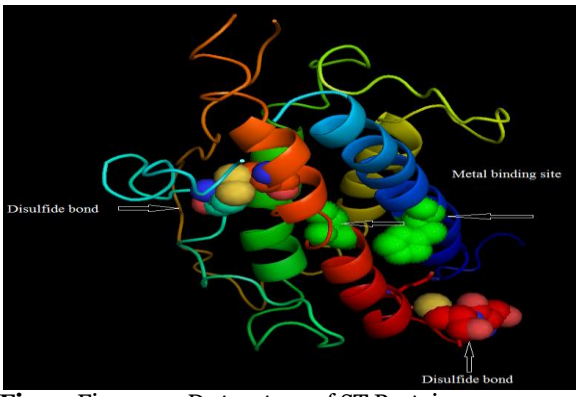

Fig. 1. Figure 1. 3D structure of ST Protein.

Multiple alignment results showed that while there are many differences in the sequences of ST protein, metal binding and disulfide bond sites are highly conserved in the all animals. A second multiple alignment also by deleting *Homo sapiens* sequence carried out. Conservation result in the second alignment was very high than first alignment which show that *Homo sapiens* sequences has more differences with the other animals (Fig 2).

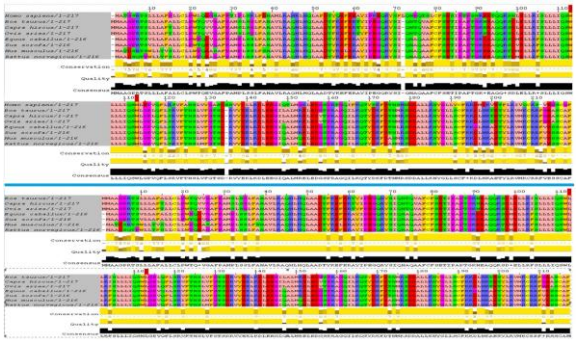

Fig. 2. Multiple Alignment of ST protein sequences; upside: all animals, Downside: all animals except Homo sapiens.

According to phylogenetic tree, selected eight animals totally classified in four groups, (a) *Equus caballus* and *Sus scrofa*, (b) *Mus musculus* and *Rattus norvegicus*, (c) *Ovis Aries*, *Bos Taurus* and *Capra Hircus and (d) Homo Sapiens*. Also, phylogenetic tree approved previous results which *Homo sapiens* ST protein sequences have more differences with other animal sequences. Based on differences of *Homo sapiens* ST with other animals, it is distinct that bST cannot used as replacement for hST.

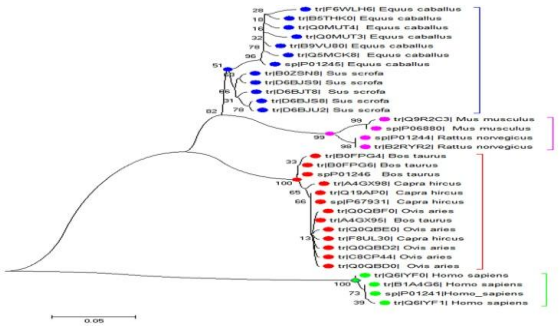

Fig. 3. Phylogenetic tree of studied animals base on ST protein.

Conclusion

Results show that based on ST protein sequences, animals in the present study divide in two groups and *Homo sapiens* did not perch in each group exactly. While metal binding and disulfide bond amino acids of *Homo sapiens* are same to other animals but, ST protein sequence of *Homo sapiens*, based on multiple alignment and phylogenetic tree results, had more different sequence in compare to other animals. Regarding to 3D structure of ST protein it's visible that disulfide bond and metal binding amino acids is very important for function of mature protein, because, any changes in these amino acids leads to bad folding of protein domain and nonfunctional protein. Differences of St protein of *Homo sapiens* finally caused other animals ST protein to be nonfunctional in humans and had no effect on growth of humans .

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