

Full Length Research Paper

Deletion of amino acid residues 33-46 in growth hormone alters the hydrophobicity of the molecule

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Growth hormone (GH) variants have been studied for the structure-function relationship of the molecule. The presence of a potential alternate splicing point in mRNA in bGH gene at exon 3, similar to hGH has been reported by workers. Early investigation on the characteristics of the chemistry of 20k oGH showed that the molecule was produced by site-directed mutagenesis by deleting amino acid residues 33-46 and the resultant DNA was expressed in *E. coli* under the control of lac promoter in pUC based plasmid. The mutant protein remained insoluble and did not refold. To investigate the effect of deletion on the chemistry of the molecule, computational biology tools were employed. The mutant with the deletion of amino acid residues 33-46, was designed and the model was visualized on computer. The structure of 20k bGH was compared with bGH and dissected for hydrogen bonds and hydrophobicity. Computational biology tools were helpful in elucidating the role of 33-46 amino acid residues domain in the chemistry of the molecule. Furthermore, it was revealed that removal of amino acid residues 33-46 which formed the hydrogen bonds involving Glu 33, Gln 46, Pro 38, Arg 42, Tyr 43, Ala 51, Thr 48, Asn 47, led to the formation of new hydrogen bonds between Thr 33, Tyr 144, Asn 32, Asn 32 and Ser and Asp 153. The removal of the amino acids 33-46 decreased the hydro-phobicity of the first helix of bGH molecule, as compared to 20k hGH, thus altering the solubility of the molecule, confirming the earlier reported results for ovine growth hormone with same deletion.

Key words: Ovine growth hormone, 20k hGH, hydro-phobicity.

INTRODUCTION

Growth hormone (GH) is a polypeptide chain which is involved in a number of anabolic processes (Wallis, 1985). A number of reports are available on the primary structure, gene cloning and expression of growth hormone gene (Santome, 1971; Sami, 2006; Sereikeite, 2007; Wallis, 1973, 1985, 2001, 2005, 2006; Verma, 1999; Yato, 1988; Vize, 1987). GH gene in ruminants comprised of 2.1 KB present at chromosome 19. Bovine GH gene (bGH) has 5 exons and 4 introns. Removal of introns is a critical step in the transcription of the protein, as any event at a potential splicing point could introduce some changes in the nucleotide sequence which could lead to the expression of a GH variant, as it happened in the case of 20k hGH (Denoto, 1981; Rogers and Walls, 1980; Lerner et al., 1980; Seif et al., 1979). Such potential event has been reported by Woychik et al. (1982) for bovine GH gene at exon 3. Another event has been reported by Hampson and Rottman (1987) in which the inclusion of exon 4 sequence has been reported in mRNA for GH. Researchers are in constant search of the

identification of different motifs in GH involved in different functions. GH has its role in a number of anabolic functions like protein synthesis, bone elongation and growth promoting activity. Availability of naturally occurring GH variants has provided some clues identifying the functions of different motifs of GH. Detection of 20k hGH could be considered as an important land mark in this connection.

Revealing of the fact that GH could be used as a tool for increasing farm animals productivity (increase in milk and meat production by the exogenous supply of GH) has made biotechnologist to pay great attention to this molecule (Jones et al., 1994; Mikel, 1993; Seve et al., 1993; Sillence, 2004; Thatcher, 2002). GH has now been successfully produced as a recombinant protein with proper folding and is being heavily used in the dairy industry. Recombinant GH has been produced as a major protein which is a recombinant DNA-derived GH molecule in the form of insoluble granules. Bioactivity of the recombinant molecule is dependent on the solubility

(A)

28 29 30 31 32 33 34 35 36 37 38 39 40
 ACC TTC AAA GAG TTT **GAG CGC ACC TAC ATC CCG GAG GGA**
 Thr Phe Lys Glu Phe **Glu Arg Thr Tyr Ile Pro Glu Gly**

41 42 43 44 45 46 47 48 49 50 51
CAG AGA TAC TCC ATC CAG AAC ACC CAG GTT GCC
Gln Arg Tyr Ser Ile Gln Asn Thr Gln Val Ala

(B)

↓

28 29 30 31 32 47 48 49 50 51
 ACC TTC AAA GAG TTT AAC ACC CAG GTT GCC
 Thr Phe Lys Glu Phe Asn Thr Gln Val Ala

Figure 1(a). Shows the nucleotides and amino acid sequence corresponding to residue 28-51. Amino acid residue planned to be deleted are shown in bold. (b). The amino acid sequence and amino acid sequence of residue 28-51, except 33-46 is shown. Arrow shows the position of deletion.

and correct folding of the molecule. For this purpose, the chemistry of the protein has been extensively studied. GH molecule has two disulfide bridges. Correct folding of the molecule is linked to the precise formation of these two bridges. (Langley et al., 1987(a, b); Storrs et al., 2001; Baranauskaitė, 2005). Tou et al. (2009) has reported the generation of two novel bGH species generated from a common dehydroalanine intermediate under stressed conditions. Borromeo et al. (2008) has studied the effects of renaturing and storage conditions of mink growth hormone. Introduction of biology computational tools has helped to uncover chemistry, structure and function relationship of GH molecules. De Vos (1992) has reported the crystal structure of the molecule. Previously, structure function studied with reference to molecular modeling has been reported revealing some important features of the molecule (Sami, 2007). Earlier a ovine growth hormone (oGH) mutant with the deletion of 32-46 amino acid residues was produced, though expressed as a major protein but was unable to refold properly and remained insoluble (Sami, 1991)

The present study was aimed to identify the role of domain comprising of 32-46 in bGH/oGH. For this purpose, computational biology tools were used and the molecule was designed and visualized. Such study for

bGH/oGH has not been reported so far.

METHODS

Plasmid oGH1 was provided for the preparation of deletion mutant 32-46 by site directed mutagenesis. Production of the mutant has been described earlier (Sami, 1991), briefly it is given below:

Production of deletion mutant 33-46 of ovine growth hormone by site directed mutagenesis

An oligonucleotide was designed to enable the production of recombinant DNA derived oGH variant equivalent to 20k hGH, involving the deletion of part of loop between helix 1 and helix 2 (Figure 1). The site directed mutagenesis was carried out according to the method as described in Amersham kit for site-directed mutagenesis using DNA sequence coding for OGH1 (Wallis and Wallis, 1988). The resultant mutant DNA was sequenced by di-deoxy sequencing method and was transferred to a pUC 8 plasmid using Eco R1 and Hind III sites. After transformation into *JM109 E. coli*, the expression of the protein was checked on SDS-PAGE. The expressed protein was isolated and refolded as described by Wallis and Wallis (1990).

The solubility and refolding of the protein was checked on SDS-PAGE. This part of research was presented in a published PhD thesis of the author submitted to University of Sussex England U K entitled Studies on recombinant DNA derived ovine growth hormone (1991) (Figure 1).

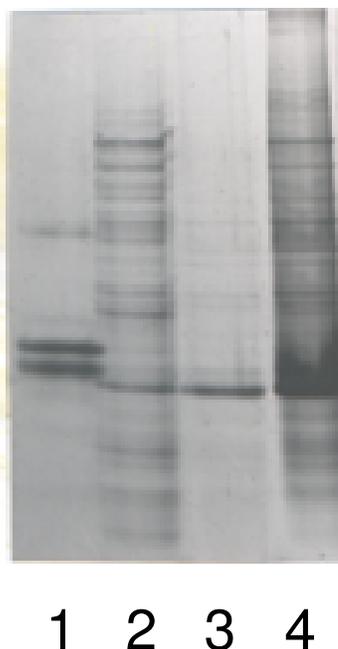


Figure 2. SDS_PAGE of total cellular proteins on 10% gel of *E.coli* containing plasmid for the expression of oGH with deletion of 33-46 amino acid residues. 1= bGH and prolactin marker; 2 = Supernatant of total cellular proteins of *E.coli* containing plasmid for the expression of oGH with deletion 33-46 amino acid residues; 3 = Pellet obtained after refolding of total cellular proteins of *E.coli* containing plasmid for the expression of oGH with deletion 33-46 amino acid residues. The variant protein visible in the pellet fraction, remained insoluble; 4 = Total cellular protein. (Source, Sami, 1991).

Computational methods

Amino acid sequence for bGH accession No. NM_18096 was used (as bGH has more than 99% homology with oGH) and amino acid 33-46 were deleted using computational tool, to make a protein equivalent to 20k bGH. Similarly, hGH accession No. AAA72260) and 20k hGH (after deleting the amino acid residues 32-46) molecules were visualized. The amino acid sequence was submitted to EXPASY server and CPH for molecular model (Arnold et al., 2006; Schwede et al., 2003; Lund et al., 2002). The model for 20k bGH and wild type bGH were superimposed, to compare the two molecules. Visualized bGH molecule appeared in green while 20k bGH appeared in pink. All the four models were visualized for hydro-phobicity by employing computer program. The hydrophobic regions appeared in blue while hydrophilic regions appeared in red. To analyze the role of hydrogen bonding for wild type and mutant bGH, hydrogen bonds were identified using a display style dialog in the view menu of program. Deleted amino acids from wild type hGH was used to make 20 k hGH, EAYIPQKYSFLQAPQ. Similarly, deleted amino acids from wild type bGH was used to make 20 k bGH, ERTYIPEGERSYSIQ.

RESULTS

Production of deletion mutant 33-46 by site directed mutagenesis

Site directed mutagenesis for 33-46 deletion mutant was

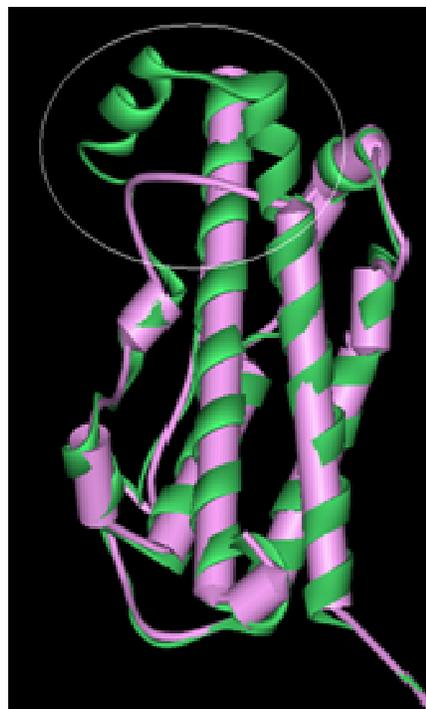


Figure 3. Molecular model of bGH (green, visible in ribbon structure) super imposed on mutant 20K bGH appeared in pink cylinders using Accelrys DS Visualizer. Previously, the model was prepared on Expasy Server. Difference in the structure of the two proteins is encircled for amino acid residues 33-46 of bGH which are absent in 20k bGH.

carried out using Amersham kit and the method was followed. The resultant DNA was sequenced and the deletion of 42 bases was noticed in the DNA sequence (Figure 1).

Ovine growth hormone variant, oGH1, with deletion of 33-46 residues was produced by site-directed mutagenesis. The protein which was produced in the form of insoluble granules was solubilized and attempted to refold, using the method employed for refolding of other ovine GH variants (Wallis and Wallis, 1990; Sami et al., 2008). The results showed that the mutant remained insoluble and did not refold (Lane 2, Figure 2).

Computational biology

Molecular model of bGH was visualized as ribbon model in green while 20k bGH molecule appeared in pink in cylinders for alpha helices. It was clearly noticed that there was no difference in the global geometry of the molecule. 20 kb GH molecule followed the twisted helical bundle form of the GH molecule arranged in left twisted fashion. The difference observed was the shortening of the first helix and the removal of the coil between helix 1 and 2. The difference is encircled in Figure 3. The

deletion of amino acid residues 33-46 did not alter the three D structure of the molecule. To further investigate the problem, the hydrophobicity of the 20k variant and the wild type bGH and hGH were studied. It was revealed that the change alter the hydrophobicity of the 20k oGH to a greater extent as compared to 20k hGH (Figures 4b and 4d). The first helix in case of 20k bGH was completely turned hydrophilic while in case of 20k hGH, the hydrophobicity was reduced as compared to wild type hGH but was not completely altered. When the chemistry of the deleted amino acid residues was compared, it was revealed that for making 20k oGH molecule at three places highly charged amino acid residues E were removed at positions 33, 39 and 41, thus converting hydrophobic core into hydrophilic region. While in case of 20k hGH only one E was removed, justifying the decrease in the hydrophobicity but not introducing the hydrophilicity, as in the case of 20k bGH molecule. Hydrogen bonds play an important role in the chemistry of the molecule. Hydrogen bonds in the region 28-56 of bGH before and after deletion were located. As shown in Figures 5a and 5b, all bonds involving Glu 33, Gln 46, Pro 38, Arg 42, Tyr 43, Ala 51, Thr 48, Asn 47, (bGH Figure 5a) were removed and new bonds were formed between Thr 33, Tyr 144, Asn 32, Asn 32 and Ser and Asp 153 (Figure 5b). These results obtained by using bioinformatics tools confirmed previously reported results for recombinant DNA derived oGH molecules having deletion of 33-46 amino acid residues (Figures 3 and 4). The loop was deleted by site-directed mutagenesis leading to the decreased solubility of the molecule with the protein being produced as insoluble granules. The recombinant protein was insoluble, as the hydrophobicity of the molecule was altered.

DISCUSSION

The Growth hormone variants were studied to identify the structure and function relationship in the molecule. 20k hGH variant has been studied in detail as it has been reported to have shown normal growth promoting activity and little insulin like activity (Lewis et al., 1986). 20k hGH is a result of mRNA splicing as it plays an important role in the production of GH and GH like proteins. The intervening sequences spliced out of the gene are transcribed to mRNA. Human GH variants such as 17.5k hGH and 20k hGH are the products of such splicing (as reviewed by Wallis, 1985). Earlier Woychick et al. (1982) has reported that bGH gene also contained a potential alternate RNA splice point at about the same position within the third exon (42 nucleotides from the second intron). The alternate site ends in AG, contains several nucleotides that are consistent with the consensus sequence of RNA splicing and has a significant homology with the U1 RNA (Seif et al., 1979; Moore et al., 1982; Lerner et al., 1980; Roger and Wall, 1980). The goal was

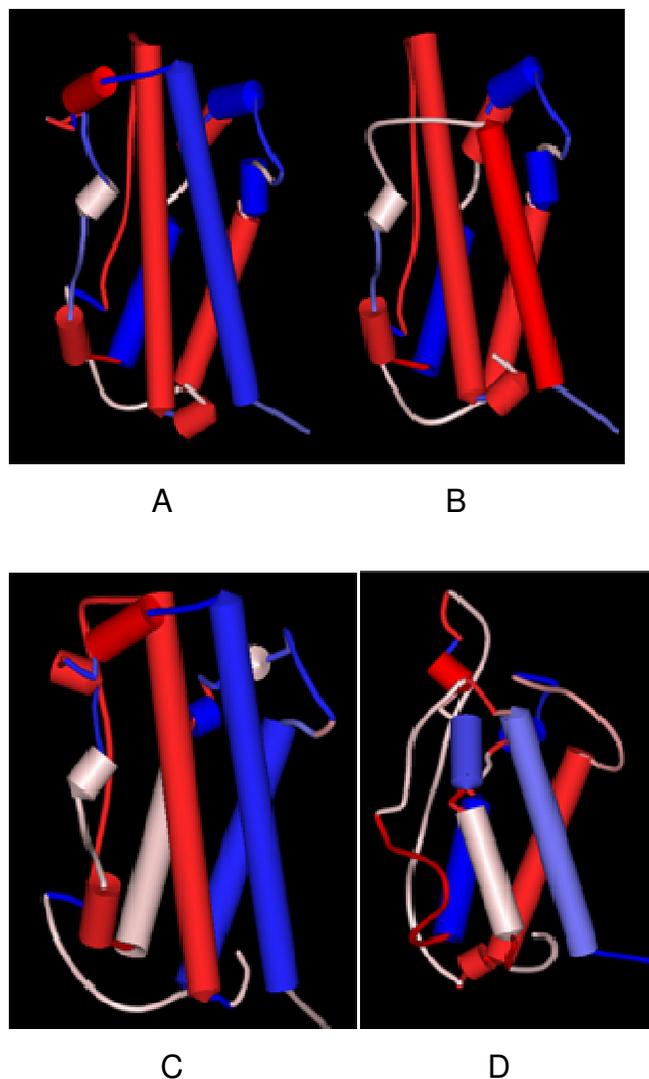


Figure 4. Molecular model of wild type bGH (A) and hGH (C), were compared with 20K hGH, deleting amino acid residues 32 - 46 (B) and 20K bGH, deleting amino acid residues 33 - 46 (D), for their mutant structure. Hydrophobicity decreased from blue to red, employed by Accelrys DS Visualizer, previously the model was prepared on Expassy Server and CPH Model. (blue colour indicates hydrophilic region while red indicated hydrophobic region of the molecule).

to determine the effects of deletion of 33-46 amino acid residues in order to relate the structure to the function of the molecule. A mutant protein for oGH1 with deletion of 33-46 amino acid residues was produced by site directed mutagenesis. The sequence analysis of mutant DNA showed that the mutant DNA had a deletion of 42 bases as compared to the original oGH1 DNA. The mutant DNA was transferred to a pUC plasmid and the expression of the protein was checked, as described previously (Wallis and Wallis, 1988; Sami et al., 2008). The mutant protein was expressed under the control of lac promoter. The mutant expressed as more or less with equal efficiency

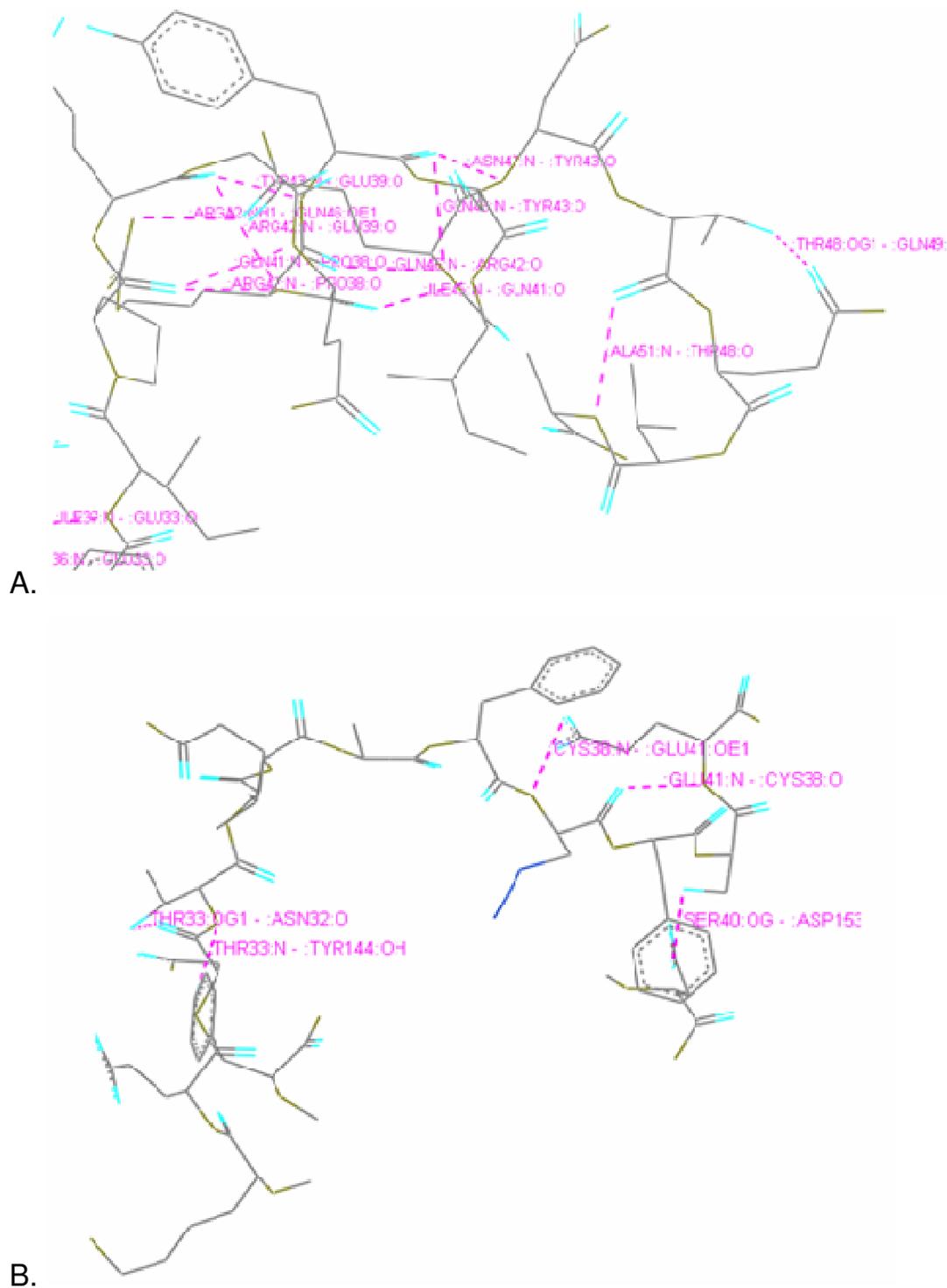


Figure 5. Hydrogen bonds identified in the region 33 - 46 amino acids in the wild type (A) and mutant (B). Hydrogen bonds appeared in the pink dotted lines.

like the original oGH1 protein expressed as major protein (Wallis and Wallis, 1989), as shown in Figure 2. The protein was solubilised in 8 M guanidine chloride and was refolded, as described previously. It was observed that

the total mutant protein did not solubilised in the buffer at pH 9.5 after treatment for refolding. It showed that the refolded protein has minimum solubility, as compared to the wild type protein. 20k bGH molecule was super-

imposed on wild type bGH and it was observed that the deletion did not alter the three dimensional structure of the molecule (Figure 4). Both models fit nicely except the part which was deleted from the molecule after the removal, of which four helices remained the initial coordinate. 20k hGH has been studied by workers (Lewis et al., 1978, 1981) and reported that 20k hGH and 22k hGH were not very different from each other in a number of bioassays but may have reduced insulin like activity. Nishikawa et al. (1989) produced 20k GH by recombinant DNA technology and reported that the mutant protein behaved like hGH in weight gain assays, deletion of a part of loop may was compensated for by the flexibility of the loop (Sami, 2007). It showed that the 20kGH produced as mutant by recombinant DNA technology methods did not alter the folding properties of the molecule.

For further investigations, as solubility of the molecules is an important factor, hydrophobicity of the molecule was checked by employing computational biology tools. It was observed that the removal of three E from the mutant altered hydrophobicity of the molecule converted the hydrophobic region into hydrophilic region (Figure 5b). While in the case of 20 k hGH deletion, the solubility of the molecule was reduced.

It may be noted that the deletion did not alter the bonds formed by Cys 53 indicating that the change did not alter the chemistry of refolding, as the hydrophobic core in GH molecule is not disturbed which provided a driving force to the molecule for refolding (Kauzman, 1959; Sami, 2007; Tsai, 2001). Perhaps 20k bGH molecule was produced as a result of mRNA alternate splicing but due to its insolubility, it may not be present in the circulation. An example which could be quoted in this regard is of 17.5k hGH, which has been reported to be the result of alternate mRNA splicing in which the mutations in the first and sixth base pair have resulted in misplacing of mRNA and loss of exon 3, so that GH produced from this message lacks amino acid residues 32-71, forming entire connecting loop (Nishikawa et al., 1989; de Vos, 1992; Lecomte et al., 1987; Sami 2007). Lecomte et al. (1987) has produced the variant by recombinant DNA technology and reported the instability of the molecule. Earlier a C-terminal deletion mutant was produced for ovine growth hormone oGH. Deleted 133-146 (loop between helix 3 and 4) did not alter the biological activity and the refolding of the molecule (Sami et al., 1999).

Conclusively, the domain comprising of amino acid residues 33-46 in bovine GH and ovine GH is involved in solubilizing the protein molecule. Its removal (as it contains highly charged amino acid residues) could change the global chemistry of the molecule by reducing its solubility.

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REFERENCES

- Arnold K, Bordoli L, Kopp J, Schwede T (2006). The SWISS-MODEL Workspace: A web-based environment for protein structure homology modeling. *Bioinformatics*, 22: 195-201.
- Baranauskaite L, Sereikaite J, Gedminiene G, Bumeliene Z, Bumelis VA, (2005). Refolding of porcine growth hormone from inclusion bodies of *Escherichia coli*. *Biocatal. Biotransformation* 23:185-189
- Borromeo V, Sereikaite J, Bumelis V, Secchi C, Scire A, Ausili A, D'Auria S, Tanfani F (2008) Mink Growth Hormone Structural-Functional Relationships: Effects of Renaturing and Storage Conditions. *Protein J.* 27: 170-180
- De Vos AM, Ultsch M, Kossiakoff AA (1992). Human growth hormone and extracellular domain of its receptor: crystal structure of the complex. *Science*, 255:306-312.
- DeNoto FM, Moore D, Goodman HM (1981). Human growth hormone DNA sequence and mRNA structure: possible alternative splicing. *Nucleic Acids Res.* 1981 August 11; 9(15): 3719-3730.
- Hampson RK, Rottman FM (1987). Alternative processing of bovine growth hormone mRNA: non-splicing of the final intron predicts a high molecular weight variant of bovine growth hormone. *Proc. Natl. Acad. Sci. USA.* 1987 May; 84: 2673-2677.
- Jones S, Schaefer A, Tong A, Robertson W, Holt L (1994). Effects of prolonged release recombinant porcine somatotropin and dietary protein on the growth, feed efficiency, carcass yield and meat quality of pigs *Can J. Anim. Sci.* 74:15-22.
- Kauzmann W (1959). Some factors in the interpretation of protein denaturation. *Adv. Protein Chem.* 14: 1-63.
- Langley KE, Berg TF, Strickland TW, Fenton DM, Boone TC, Wypych J (1987). Recombinant-DNA-derived bovine growth hormone from *Escherichia coli*. 1. Demonstration that the hormone is expressed in reduced form, and isolation of the hormone in oxidized, native form *Eur. J. Biochem/FEBS* 163(2):313-321.
- Langley KE, Lai PH, Wypych J, Everett RR, Berg TF, Krabill LF, Davis JM, Souza LM (1987). Recombinant-DNA-derived bovine growth hormone from *Escherichia coli*. 2. Biochemical, biophysical, immunological and biological comparison with the pituitary hormone *Eur. J. Biochem/FEBS* 163: 323-330.
- Lecomte CM, Renard A, Martial IA (1987). A new natural hGH variant-17.5 Kd produced by alternative splicing. An additional consensus sequence which might play a role in branchpoint selection. *Nucl. Acid. Res.* 15: 6331-6348.
- Lerner MR, Boyle JA, Mount SM, Wolin SL, Steitz JA (1980). Are snRNPs involved in splicing? *Nature.* 283: 220-224.
- Lewis UJ, Bonewald LF, Lewis LJ (1980). The 20,000-dalton variant of human growth hormone: location of the amino acid deletions. *Biochem. Biophys. Res. Commun.* 92(2):511-516.
- Lewis UJ, Dunn JT, Bonewald LF, Seavey BK, Vanderlaan WPA (1978). Naturally occurring structural variant of human growth hormone. *J. Biol. Chem.* 253: 2679-2687.
- Lund OM, Nielsen C, Lundegaard, Worning P (2002). CPH models CP 2.0: X3M a Computer Program to extract 3D Models. Abstract at the CASP5 conference A102.
- Mikel W, Althen T, Rogers R, Moore A, Miller H, Miller L (1993) Effects of exogenous porcine somatotropin on the carcass composition, hormonal and metabolic profiles, lipogenic capacity, and binding of insulin to erythrocyte receptors of fast- versus slow-growing swine *J. Anim. Sci.* 71: 1786-1795.
- Moore JA, Pletcher SA, Ross MJ (1986) Absorption enhancement of growth hormone from the gastrointestinal tract of rats *Int. J. Pharm.* 34:35-43.
- Nishikawa S, Nishida Y, Uemura H, Yamada Y, Tanaka T, Uesugi S, Morikawa M, Uchida E, Hayakawa T, Ikehara M (1989) Structure and activity of artificial mutant variants of human growth hormone *Protein Eng.* 3: 49-53.
- Rogers J, Wall RA (1980). Mechanism for RNA splicing. *Proc. Natl. Acad. Sci. USA.* 77:1877-1879.
- Sami AJ (1991). PhD thesis Studies on recombinant DNA derived Ovine growth hormone. University of Sussex England UK.

- Sami AJ (2006). Purification of GH from local specie of water buffalo *Bubalus bubalis* Pak. J. Zool. 38: 279-282.
- Sami AJ (2007). Structure-function relation of somatotropin with reference to molecular modeling. Current Protein and Peptide Science, 8: 283-292.
- Sami AJ, Wallis OC, Wallis M (1999). Production and characterization of deletion mutants of ovine growth hormone J. Mol. Endocrinol. 23: 97-106.
- Sami AJ., Wallis OC, Wallis M (2008). Production, purification and characterization of two recombinant DNA-derived N-terminal ovine growth hormone variants: oGH3 and oGH5. Afr. J. Biotechnol. 7: 1859-1864.
- Santome JA, Dellacha JM, Paladini AC, Wolfenstein CEM, Pena C, Poskus E, Daurat ST, Biscoglio MJ, De Sese ZMM, De Sanguesa AVF (1971). Amino acid sequence of bovine growth hormone FEBS Lett 16:198–200.
- Schwede T, Kopp J, Guex N, and Peitsch MC (2003). SWISS-MODEL: An automated protein homology-modeling server. Nucleic Acids Res. 31: 3381-3385.
- Seif I, Khoury G, Dhar R (1979). BKV splice sequences based on analysis of preferred donor and acceptor sites. Nucleic Acids Res. 6:3387–3398.
- Sereikaite J, Statkute A, Morkunas M, Radzevicius K, Borromeo V, Secchi C, Bumelis VA (2007). Production of recombinant mink growth hormone in *E. coli* Appl. Microbiol. Biotechnol. 74: 316–323.
- Seve B, Balleve O, Ganier P, Noblet J, Prugnaud J, Obled C (1993). Recombinant porcine somatotropin and dietary protein enhance protein synthesis in growing pigs J. Nutr. 123:529–540.
- Sillence MN (2004). Technologies for the control of fat and lean deposition in livestock. Vet. J. 167:242–257.
- Storrs SB, Tou JS, Ballinger JM (2001). Method for solubilization and naturation of somatotropins, US 6,034,224.
- Thatcher CE, Thompson DL Jr (2002). Effects of frequency of treatment with recombinant equine somatotropin on selected biological responses in geldings. Domest. Anim. Endocrinol. 22:127–143.
- Tou JS, Violand BN, Che ZY, Carrol JA, Schlitter MR, Egodange K, Poruthoor S, Lipartito C, Basler DA, Cagney JW, Storrs SB (2009). Two novel bovine somatotropin species generated from a common dehydroalanine intermediate. Protein J. 28(2):87-95.
- Tsai C, Nussinov R (2001). The building block folding model and the kinetics of protein folding. Protein Eng. 14: 723-733.
- Verma S, Ghorpade A, Tiwari G, Das P, Garg LC (1999). cDNA cloning and sequence analysis of bubaline growth hormone. DNA Seq. 10:101-103.
- Vize PD, Wells JRE (1987). Isolation and characterization of the porcine growth hormone gene. Gene. 55: 339-344.
- Wallis M (1973). Primary structure of bovine growth hormone. FEBS Lett. 35:11–14.
- Wallis M, Howell SL, Taylor KW (1985). In: The Biochemistry of the Polypeptide Hormones. Wiley, Chichester pp. 184-255.
- Wallis OC, Bill LJ, Ellis SA, Wallis M (2006). Polymorphism of the growth hormone gene of red deer (*Cervus elaphus*). Gen. Comp. Endocrinol. 146: 180-185.
- Wallis OC, Maniou Z, Wallis M (2005). Cloning and characterization of the gene encoding growth hormone in finback whale (*Balaenoptera physalus*). Gen. Comp. Endocrinol. 143: 92-97.
- Wallis OC, Wallis M (1990). Purification and properties of a recombinant DNA-derived ovine growth hormone analogue (oGH1) expressed in *Escherichia coli*. J. Mol. Endocrinol. 4: 61-69.
- Wallis OC, Wallis M (2001). Molecular Evolution of Growth Hormone (GH) in Cetartiodactyla: Cloning and Characterization of the Gene Encoding GH from a Primitive Ruminant, the Chevrotain (*Tragulus javanicus*). 123: 162-172.
- Woychik RP, Camper SA, Lyons RH, Horowitz S, Goodwin EC, Rottman FM (1982). Cloning and nucleotide sequencing of the bovine growth hormone gene. Nucleic Acids Res. 10: 7197–7210.
- Yato M, Yamano Y, Oyabayashi K, Okuno M, Kioka N, Manabe E, Hashi H, Sakai H, Komano T, Utsumi K (1988). Nucleotide sequence of the growth hormone gene cDNA from goat *Capra hircus* L. (Tokara). Nucleic Acids Res. 16: 3578.