CLAIMS

1. A human chorionic gonadotropin carboxy terminal peptide (CTP)-modified human growth hormone (hGH) polypeptide, said polypeptide consisting of two CTPs attached to the carboxy terminus of the hGH and one CTP attached to the amino terminus of the hGH, wherein the amino acid sequence of said polypeptide is set forth in SEQ ID NO: 7, and wherein the CTP-modified hGH polypeptide comprises glycosylation at between 13 and 18 O-linked glycosylation sites.

2. The human CTP-modified hGH polypeptide according to claim 1, wherein said glycosylation occurs on the CTP units and there is no glycosylation of the hGH.

3. The human CTP-modified hGH polypeptide according to any one of the preceding claims, wherein each CTP contains 4, 5, or 6 O-linked glycans.

4. The human CTP-modified hGH polypeptide according to any one of the preceding claims, wherein the glycosylation of said CTP units comprises at least 15 O-linked glycans.

5. The human CTP-modified hGH polypeptide according to any one of the preceding claims, wherein the human CTP-modified hGH polypeptide comprises O-linked glycosylation of residues 10, 13, 15, 21, 229, 232, 234, 240, 257, 260, 262, and 268 of SEQ ID NO: 7.

6. The human CTP-modified hGH polypeptide according to claim 5, wherein the pattern of said glycosylation the human CTP-modified hGH polypeptide further comprises O-linked glycosylation of one or two residues selected from amino acid residues 1-4, one or two residues selected from amino acid residues 220-223, one or two residues selected from amino acid residues or 248-251, of SEQ ID NO: 7, or a combination thereof.

7. The human CTP-modified hGH polypeptide according to any one of the preceding claims, wherein the O-glycan structures comprise sialylated core structures.

8. The human CTP-modified hGH polypeptide according to any one of the preceding claims, wherein the glycosylation at each of said O-linked glycosylation sites comprises a glycan core structure selected from the group comprising a mono-sialylated core 1 (Neu5Acα2- 3Galβ1-3GalNAc), a neutral core 1 (Galβ1-3GalNAc), a mono-sialylated core 1 (Neu5Acα2-6(Galβ1-3)GalNAc), or a di-sialylated core 1 (Neu5Acα2-3Galβ1- 3(Neu5Acα2-6)GalNAc).

9. A composition comprising the CTP-modified hGH polypeptide according to any one of the preceding claims, and a pharmaceutically acceptable carrier.

10. The composition according to claim 9, wherein at least about 90% of the CTP-modified hGH polypeptides present in the composition are glycosylated with between 13-18 O-glycan occupancy.

11. The composition according to any one of claims 9-10, wherein the most abundant form of glycosylated CTP-modified hGH polypeptide present in the composition comprises 15 O-glycans.

12. The composition according to any one of claims 9-11, wherein at least about 60% of the CTP-modified hGH polypeptides present in the composition are sialylated.

13. The composition according to any one of claims 9-12, wherein the CTP-modified hGH polypeptide present in the composition comprises an O-glycan structure with a mono-sialylated core 1 (Neu5Acα2-3Galβ1-3GalNAc).

14. The composition according to any one of claims 10-13, wherein the major O-glycan in the composition is a mono-sialylated core 1 (Neu5Acα2-3Galβ1-3GalNAc)

15. The composition according to any one of claims 12-14, wherein the major sialic acid in the composition is Neu5Ac.

16. The composition as claimed in any one of claims 9-15, comprising one or more of the following characteristics

    i. less than or equal 100 ng/mg (ppm) of host cell proteins;

    ii. less than or equal to 10 pg/mg DNA;

    iii. less than 50 ng/mL of methotrexate;

    iv. less than or equal 60 µg/ml propylene glycol;

    v. less than or equal 2.5 µg/mL Triton;

    vi. less than or equal 115 pg/mL insulin;

    vii. less than 250 µg/mL DMSO; or

    viii. less than or equal 10 cfu/10 mL in a Bioburden assay.

17. The human chorionic gonadotropin carboxy terminal peptide (CTP)-modified human growth hormone (hGH) polypeptide according to any one of claims 1-8, or the composition according to any one of claims 9-16, manufactured by a method comprising the steps of:

(a) stably transfecting a predetermined number of cells with an expression vector comprising a coding portion encoding said CTP-modified hGH; wherein said transfected cell expresses and secretes said CTP-modified hGH;

(b) obtaining cell clones that overexpress said CTP-modified hGH;

(c) expanding said clones in solution to a predetermined scale;

(d) harvesting said solution containing said clones;

(e) filtering said solution containing said clones to obtain a clarified harvest solution; and,

(f) purifying said clarified harvest solution to obtain a purified protein solution having a desired concentration of a CTP-modified hGH;

wherein said polypeptide consists of two CTPs attached to the carboxy terminus of the hGH and one CTP attached to the amino terminus of the hGH, and wherein said manufactured CTP-modified hGH comprises the amino acid sequence set forth in SEQ ID NO: 7.

18. A method of manufacturing the human chorionic gonadotropin carboxy terminal peptide (CTP)-modified human growth hormone (hGH) polypeptide according to any one of claims 1-8, or the composition according to any one of claims 9-16, the method comprising the steps of:

(a) stably transfecting a predetermined number of cells with an expression vector comprising a coding portion encoding said CTP-modified hGH; wherein said transfected cell expresses and secretes said CTP-modified hGH;

(b) obtaining cell clones that overexpress said CTP-modified hGH;

(c) expanding said clones in solution to a predetermined scale;

(d) harvesting said solution containing said clones;

(e) filtering said solution containing said clones to obtain a clarified harvest solution; and,

(f) purifying said clarified harvest solution to obtain a purified protein solution having a desired concentration of the CTP-modified hGH;

thereby manufacturing a CTP-modified hGH, wherein the amino acid sequence of the manufactured CTP-modified hGH is set forth in SEQ ID NO: 7.

19. The method according to claim 18, wherein said purification (step f) of said clarified harvest comprises sequentially performing the following steps comprising:

(g) concentrating and diafiltering said clarified harvest solution;

(h) obtaining said clarified harvest obtained following step (g) and inactivating viruses present in said clarified harvest by incubating in a solution toxic to said viruses;

(i) obtaining said clarified harvest solution from step (h) and purifying said clarified harvest solution,

i. wherein said purifying is accomplished by sequentially passing said clarified harvest solution through an anion exchange column and a hydrophobic interaction column followed by a concentrating and diafiltering step,

ii. wherein said purifying is followed by sequentially passing said clarified harvest solution through a Hydroxyapatite Mixed-Mode column and a cation exchange column;

(j) obtaining said clarified harvest solution following step (ii) and physically removing said clarified harvest solution from viruses by nanofiltration;

(k) obtaining said clarified harvest solution following step (j) and concentrating and diafiltering said clarified harvest solution to arrive at a maximally purified clarified harvest containing said CTP-modified hGH.

20. The use of:

the CTP-modified hGH according to any one of claims 1-8 or 17,
the composition according to any one of claims 9-16, or
the CTP-modified hGH made according to the method of claim 18 or 19,
in the manufacture of a medicament for treating a subject requiring treatment with hGH.