does not fold efficiently during recombinant expression, and tends to form insoluble precipitates known as inclusion bodies. These inclusion bodies must be solubilized and functional protein must be renatured from the misfolded aggregate, a time-consuming, inefficient, and expensive process. Accordingly, there remains a need for growth hormone compositions that can increase the half-life and can be administered less frequently, but are safer and less complicated and costly to produce.

SUMMARY OF THE INVENTION

[0010] The present disclosure is directed to compositions and methods that can be useful for or the treatment of any disease, disorder or condition that is improved, ameliorated, or inhibited by the administration of growth hormone. In particular, the present invention provides compositions of fusion proteins comprising one or more extended recombinant polypeptides with a non-repetitive sequence and/or unstructured conformation (XTEN) linked to growth hormone (GH). In part, the present disclosure is directed to pharmaceutical compositions comprising the fusion proteins and the uses thereof for treating growth hormone-related diseases, disorders or conditions.

[0010a] In one aspect, there is provided an isolated fusion protein, comprising a growth hormone (GH) that is at least 90% identical to the human growth hormone amino acid sequence shown in Table 1, wherein said growth hormone is linked to an extended recombinant polypeptide (XTEN) of at least 200 amino acids residues, wherein the XTEN is characterized in that:

(a) the XTEN sequence comprises at least 200 contiguous amino acids that exhibits at least 90% sequence identity to a comparable length of an amino acid sequence selected from Table 3;

(b) the XTEN sequence lacks a predicted T-cell epitope when analyzed by TEPITOPE algorithm, wherein the TEPITOPE algorithm prediction for epitopes within the XTEN sequence is based on a score of -6 or greater;

(c) it has a subsequence score of less than 10;

(d) the sum of glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P) residues constitutes more than 90% of the total amino acid residues of the XTEN; and

(e) the XTEN sequence is substantially non-repetitive such that (i) at least 80% of the XTEN sequence consists of non-overlapping sequence motifs wherein each of the sequence motifs has 9 to 14 amino acid residues and wherein the sequence of any two contiguous amino acid residues does not occur more than twice in each of the sequence motifs; or (ii) the XTEN sequence contains no three contiguous amino acids that are identical unless the amino acids are serine residues, in which case no more than three contiguous amino acids are serine.

[0010b] In one aspect, there is provided a pharmaceutical composition comprising the isolated fusion protein as defined herein, and a pharmaceutically acceptable carrier.

[0010c] In one aspect, there is provided a method of producing a fusion protein comprising a growth hormone (GH) fused to one or more extended recombinant polypeptides (XTEN), comprising:

(a) providing host cell comprising a recombinant polynucleotide molecule encoding the fusion protein as defined herein;

(b) culturing the host cell under conditions permitting the expression of the fusion protein; and

(c) recovering the fusion protein.

[0010d] In one aspect, there is provided an isolated nucleic acid comprising a nucleotide sequence encoding the fusion protein as defined herein or the complement thereof.

[0010e] In one aspect, there is provided an expression vector comprising the nucleic acid as defined herein.

[0010f] In one aspect, there is provided an isolated host cell comprising the nucleic acid as defined herein.

[0010g] In one aspect, there is provided a fusion protein comprising a growth hormone (GH) for treating a growth-hormone related condition in a subject, comprising the fusion protein as defined herein.

[0010h] In one aspect, there is provided a kit, comprising the pharmaceutical composition as defined herein, a label identifying the pharmaceutical composition, and an instruction for storage, reconstitution and/or administration of the pharmaceutical compositions to a subject.

[0010i] In one aspect, there is provided a fusion protein, comprising an amino acid sequence that is at least 90% identical to the amino acid sequence of AE912-hGH-AE144 as shown in Table 36.

[0010j] In one aspect, there is provided a fusion protein, comprising the amino acid sequence of AE912-hGH-AE144 as shown in Table 36.

[0010k] In one aspect, there is provided the use of a therapeutically effective amount of a fusion protein as defined herein, in the manufacture of a medicament for treating a growth-hormone related condition in a subject.

[0011] In one embodiment, the invention provides an isolated fusion protein, comprising a growth hormone that is at least about 90%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99% identical to an amino acid sequence selected from Table 1, wherein said growth hormone is linked to an extended recombinant polypeptide (XTEN) of at least about 100, or at least about 200, or at least about 400, or at least about 800, or at least about 900, or at least about 1000, or at least about 2000, up to about 3000 amino acids residues, wherein the XTEN is characterized in that (a) the XTEN comprises at least about 200 contiguous amino acids that exhibits at least about 90%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99% identical to a comparable length of an amino acid sequence selected from a sequence shown in Table 3; (b) the XTEN sequence lacks a predicted T-cell epitope when analyzed by TEPITOPE algorithm, wherein the TEPITOPE algorithm prediction for epitopes within the XTEN sequence is based on a score of -5, or -6, or -7, or -8, or –9 or greater; (c) the XTEN has a subsequence score of less than 10, or less than 9, or less than 8, or less than 7, or less than 6, or less than 5, or even less; and (d) the sum of glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P) residues constitutes more than about 90%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99% of the total amino acid residues of the XTEN. In one embodiment, the growth hormone of the isolated fusion protein is human growth hormone. In another embodiment, the isolated fusion protein comprises at least a second XTEN, wherein the fusion protein adopts a multiple-XTEN configuration shown in Table 5, or a variant thereof.

[0012] In another embodiment, the XTEN sequence of the GHXTEN fusion proteins is characterized in that is has greater than 90% random coil formation, or about 95%, or about 96%, or about 97%, or about 98%, or about 99% random coil formation as determined by GOR algorithm; and the XTEN sequence has less than 2% alpha helices and 2% beta-sheets as determined by the Chou-Fasman algorithm.

[0013] In another embodiment, the invention provides GHXTEN fusion proteins, wherein the XTEN is characterized in that the sum of asparagine and glutamine residues is less than 10% of the total amino acid

WHAT IS CLAIMED IS:

1. An isolated fusion protein, comprising a growth hormone (GH) that is at least 90% identical to the human growth hormone amino acid sequence shown in Table 1, wherein said growth hormone is linked to an extended recombinant polypeptide (XTEN) of at least 200 amino acids residues, wherein the XTEN is characterized in that:

(a) the XTEN sequence comprises at least 200 contiguous amino acids that exhibits at least 90% sequence identity to a comparable length of an amino acid sequence selected from Table 3;

(b) the XTEN sequence lacks a predicted T-cell epitope when analyzed by TEPITOPE algorithm, wherein the TEPITOPE algorithm prediction for epitopes within the XTEN sequence is based on a score of -6 or greater;

(c) it has a subsequence score of less than 10;

(d) the sum of glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P) residues constitutes more than 90% of the total amino acid residues of the XTEN; and

(e) the XTEN sequence is substantially non-repetitive such that (i) at least 80% of the XTEN sequence consists of non-overlapping sequence motifs wherein each of the sequence motifs has 9 to 14 amino acid residues and wherein the sequence of any two contiguous amino acid residues does not occur more than twice in each of the sequence motifs; or (ii) the XTEN sequence contains no three contiguous amino acids that are identical unless the amino acids are serine residues, in which case no more than three contiguous amino acids are serine.

2. The isolated fusion protein of claim 1, wherein the growth hormone is the human growth hormone amino acid sequence shown in Table 1.

3. The isolated fusion protein of claim 1 or 2, further comprising a second XTEN sequence, and wherein the fusion protein adopts a multiple -XTEN configuration shown in Table 5.

4. The isolated fusion protein of any one of claims 1 to 3, wherein the growth hormone peptide and the XTEN is linked via a spacer, wherein the spacer sequence comprises between 1 to 50 amino acid residues, and wherein the spacer optionally comprises a cleavage sequence.

5. The isolated fusion protein of any of claims 1 to 4, wherein the fusion protein binds to the same target receptor of the corresponding native growth hormone that lacks the XTEN, and wherein said fusion protein retains at least 0.1%-30% of the binding affinity of the corresponding growth hormone that lacks the XTEN.

6. The isolated fusion protein of any of claims 1 to 5, comprising an amino acid sequence that has at least 90% sequence identity to an amino acid sequence selected from Table 35, Table 36, and Table 37.

23. The fusion protein of claim 22, wherein the growth-hormone related condition is selected from growth-hormone deficiency, Turner's Syndrome, Prader-Willi Syndrome, idiopathic short stature, AIDS wasting, multiple sclerosis, Crohn's disease, ulcerative colitis, and muscular dystrophy.

24. The fusion protein of claim 22 or 23, wherein a therapeutically effective amount of the fusion protein results in maintaining blood concentrations of the fusion protein within a therapeutic window for the fusion protein at least three-fold longer compared to the corresponding native growth hormone that lacks the XTEN administered at a comparable amount to a subject.

25. The fusion protein of any of claims 22 to 24, wherein two or more consecutive doses of the fusion protein administered under a therapeutically effective dose regimen results in a gain in time between consecutive C_{max} peaks and/or C_{min} troughs for blood levels of the fusion protein compared to the corresponding growth hormone not linked to the fusion protein and administered using a therapeutically effective dose regimen established for the GH.

26. The fusion protein of any of claims 22 to 25, wherein

(i) a smaller molar amount of the fusion protein in comparison to the corresponding growth hormone that lacks the XTEN achieves a comparable therapeutic effect as the corresponding growth hormone that lacks the XTEN;

(ii) the fusion protein is administered less frequently to a subject in comparison to the corresponding growth hormone that lacks the XTEN administered to a subject using an otherwise equivalent molar dose, and the fusion protein achieves a comparable therapeutic effect as the corresponding growth hormone that lacks the XTEN; or

(iii) an accumulatively smaller molar amount of the fusion protein is administered to a subject in comparison to the corresponding growth hormone that lacks the XTEN administered to a subject under the otherwise equivalent dose period, the fusion protein achieves a comparable therapeutic effect as the corresponding growth hormone that lacks the XTEN.

27. The fusion protein of claim 26, wherein the therapeutic effect is a measured parameter selected from IGF- 1 concentrations, IGFBP3 concentration, height velocity, lean body mass, total body fat, trunk fat, response to insulin challenge, rate of division of chondrocytes, chondrocyte numbers, bone density, bone growth, and increase in epiphyseal plate width.

28. A kit, comprising the pharmaceutical composition of claim 7, a label identifying the pharmaceutical composition, and an instruction for storage, reconstitution and/or administration of the pharmaceutical compositions to a subject.

29. A fusion protein, comprising an amino acid sequence that is at least 90% identical to the amino acid sequence of AE912-hGH-AE144 as shown in Table 36.