

[DESCRIPTION]

[Invention Title]

TRIPLE GLUCAGON/GLP-1/GIP RECEPTOR AGONIST

[Technical Field]

The present invention relates to a triple agonist having activities to all of glucagon, GLP-1, and GIP receptors, and uses thereof.

[Background Art]

Obesity and diabetes including type 2 diabetes are representative metabolic diseases that occur in modern society. These diseases are regarded as health-threatening factors in the world and the accompanying economic costs due to the incidence of these diseases are rapidly increasing at present.

Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP) are representative gastrointestinal hormones and neuronal hormones, and are materials involved in the control of blood glucose levels according to food intake. Glucagon is a peptide hormone secreted by the pancreas and is involved in controlling the blood glucose levels along with the two materials described above.

GLP-1 is a hormone secreted by the small intestine stimulated by food intake. GLP-1 promotes insulin secretion in the pancreas in a blood glucose-dependent manner and inhibits the secretion of glucagon, thus helping the action of lowering blood glucose levels. Additionally, GLP-1 has the roles of slowing digestive action in the gastrointestinal tract by acting as a satiety factor, and reducing the amount of food intake by delaying the time for emptying digested food in the gastrointestinal tract. Furthermore, the administration of GLP-1 to rats was reported to have effects of inhibiting food intake and reducing body weight, and these effects were confirmed to occur equally both in normal and obese states, thus showing the potential of GLP-1 as an agent for treating obesity.

GIP, one of the gastrointestinal hormones secreted by the stimulation of food intake,

as is the case of GLP-1, is a hormone consisting of 42 amino acids secreted by the intestinal K-cells. GIP was reported to perform the functions of promoting the secretion of insulin in the pancreas in a blood glucose-dependent manner and helping to lower the blood glucose levels, thereby exhibiting the effects of increasing the activation of GLP-1, anti-inflammation, *etc.*

Glucagon is produced in the pancreas when the blood glucose levels fall due to reasons such as medications, diseases, deficiency in hormones or enzymes, *etc.* Glucagon sends a signal for glycogen breakdown in the liver to induce the release of glucose and increases blood glucose levels to a normal level. In addition to the effect of increasing the blood glucose levels, glucagon suppresses appetite in animals and humans and activates hormone-sensitive lipase of adipocytes to promote lipolysis and energy expenditure, thereby showing an anti-obesity effect.

As such, active studies are being conducted to develop GLP-1 as a therapeutic agent for treating diabetes and obesity, based on the effects of GLP-1 controlling blood glucose levels and reducing body weight. Currently, exendin-4, prepared from lizard venom and having an amino acid homology of about 50% with GLP-1, is under development as a therapeutic agent for treating the same kinds of diseases. However, the therapeutic agents containing GLP-1 and exendin-4 were reported to show side-effects such as vomiting and nausea (Syed YY., *Drugs*, 2015 Jul; 75 (10): 1141 - 52).

Additionally, for the maximization of body weight reduction and as an alternative to the above-described GLP-1-based therapeutic material, studies have been focused on dual agonists having activities to both GLP-1 receptors and glucagon receptors, and they were shown to be more effective in body weight reduction due to the activation of glucagon receptors, compared to when the existing GLP-1 was treated alone (Jonathan W *et al.*, *Nat Chem Bio.*, 2009 Oct (5); 749 - 757).

Additionally, in the study related to triple agonists, which have activities to all of GLP-1, GIP, and glucagon receptors simultaneously, efforts have been made recently to increase the half-life of the triple agonists by substituting an amino acid sequence to increase

the resistance to dipeptidyl peptidase-IV (DPP-IV), which decomposes gastrointestinal hormones to get rid of their activities, followed by adding an acyl group to a particular region thereof (Finan B *et al.*, *Nat Med.*, 2015 Jan; 21 (1): 27 - 36). However, their effects of activating three different kinds of receptors were not significant and no triple agonist showed various active ratios thereto.

Accordingly, there is a need for the development of a novel material which can highly activate GLP-1, GIP, and glucagon receptors and has the effects of controlling blood glucose levels and reducing body weight without causing any side-effects such as vomiting and nausea.

Additionally, there is also a need for the development of a novel material which has various active ratios to GLP-1, GIP, and glucagon receptors. For example, there is an increasing need for the development of a material which has an effect of reducing body weight but has a significantly higher effect of controlling blood glucose levels due to high GLP-1 and GIP activities but with relatively low glucagon activity for a hypoglycemic effect; or a material which has high activities for all of GLP-1, GIP, and glucagon, thus having a significantly high effect of reducing body weight.

[Summary]

[Technical Problem]

An object of the present invention is to provide an isolated peptide having activities to a glucagon receptor, a glucagon-like peptide-1 (GLP-1) receptor, and a glucose-dependent insulinotropic polypeptide (GIP) receptor.

Another object of the present invention is to provide a polynucleotide encoding the isolated peptide, a recombinant expression vector including the polynucleotide, and a transformant including the polynucleotide or recombinant expression vector.

Still another object of the present invention is to provide a method for preparing the isolated peptide.

Still another object of the present invention is to provide a composition containing the isolated peptide.

Still another object of the present invention is to provide a method for treating a

target disease, which includes administering the isolated peptide or a composition containing the isolated peptide to a subject in need thereof.

Still another object of the present invention is to provide a use of the isolated peptide or the composition for use in the preparation of a medicament.

[Technical Solution]

To achieve the above objects, in an example, the present disclosure provides an isolated peptide having activities to a glucagon receptor, a glucagon-like peptide-1 (GLP-1) receptor, and a glucose-dependent insulinotropic polypeptide (GIP) receptor.

In one aspect, the present disclosure refers to an isolated peptide having the function of a triple agonist which activates a glucagon receptor, a glucagon-like peptide-1 (GLP-1) receptor, and a glucose-dependent insulinotropic polypeptide (GIP) receptor, wherein the isolated peptide comprises an amino acid sequence represented by General Formula 1 below:

Xaa1-Xaa2-Xaa3-Gly-Thr-Phe-Xaa7-Ser-Asp-Xaa10-Ser-Xaa12-Xaa13-Xaa14-Xaa15-Xaa16-Xaa17-Xaa18-Xaa19-Xaa20-Xaa21-Phe-Xaa23-Xaa24-Trp-Leu-Xaa27-Xaa28-Xaa29-Xaa30-R1 (General Formula 1, SEQ ID NO: 103)

wherein, in General Formula 1,

Xaa1 is histidine (His, H), 4-imidazoacetyl (CA), or tyrosine (Tyr, Y);

Xaa2 is glycine (Gly, G), or aminoisobutyric acid (Aib);

Xaa3 is glutamic acid (Glu, E) or glutamine (Gln, Q);

Xaa7 is threonine (Thr, T) or isoleucine (Ile, I);

Xaa10 is leucine (Leu, L), tyrosine (Tyr, Y), lysine (Lys, K), cysteine (Cys, C), or valine (Val, V);

Xaa12 is lysine (Lys, K), serine (Ser, S), or isoleucine (Ile, I);

Xaa13 is glutamine (Gln, Q), tyrosine (Tyr, Y), alanine (Ala, A), or cysteine (Cys, C);

Xaa14 is leucine (Leu, L), methionine (Met, M), or tyrosine (Tyr, Y);

Xaa15 is cysteine (Cys, C), aspartic acid (Asp, D), glutamic acid (Glu, E), or leucine (Leu, L);

Xaa16 is glycine (Gly, G), glutamic acid (Glu, E), or serine (Ser, S);

Xaa17 is glutamine (Gln, Q), arginine (Arg, R), isoleucine (Ile, I), glutamic acid (Glu, E), cysteine (Cys, C), or lysine (Lys, K);

Xaa18 is alanine (Ala, A), glutamine (Gln, Q), arginine (Arg, R), or histidine (His, H);

Xaa19 is alanine (Ala, A), glutamine (Gln, Q), cysteine (Cys, C), or valine (Val, V);

Xaa20 is lysine (Lys, K), glutamine (Gln, Q), or arginine (Arg, R);

Xaa21 is glutamic acid (Glu, E), glutamine (Gln, Q), leucine (Leu, L), cysteine (Cys, C), or aspartic acid (Asp, D);

Xaa23 is valine (Val, V);

Xaa24 is alanine (Ala, A), glutamine (Gln, Q), cysteine (Cys, C), asparagine (Asn, N), aspartic acid (Asp, D), or glutamic acid (Glu, E);

Xaa27 is valine (Val, V), leucine (Leu, L), or lysine (Lys, K);

Xaa28 is cysteine (Cys, C), lysine (Lys, K), alanine (Ala, A), asparagine (Asn, N), or aspartic acid (Asp, D);

Xaa29 is cysteine (Cys, C), glycine (Gly, G), glutamine (Gln, Q), threonine (Thr, T), glutamic acid (Glu, E), or histidine (His, H);

Xaa30 is cysteine (Cys, C), glycine (Gly, G), lysine (Lys, K), or histidine (His, H), or is absent; and

R1 is cysteine (Cys, C), GKNDWKHNIT (SEQ ID NO: 106), m-SSGAPPPS-n (SEQ ID NO: 107), or m-SSGQPPPS-n (SEQ ID NO: 108), or is absent;

wherein:

m is -Cys-, -Pro-, or -Gly-Pro-; and

n is -Cys-, -Gly-, -Ser-, or -His-Gly-, or is absent.

In one example, the present disclosure refers to an isolated peptide having activities to a glucagon receptor, a glucagon-like peptide-1 (GLP-1) receptor, and a glucose-dependent insulinotropic polypeptide (GIP) receptor.

In another aspect, the present disclosure refers to an isolated peptide having the function of a triple agonist which activates a glucagon receptor, a glucagon-like peptide-1 (GLP-1) receptor, and a glucose-dependent insulinotropic polypeptide (GIP) receptor, wherein the isolated peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 1 to 102.

In another aspect, the present disclosure refers to a polynucleotide encoding the peptide as disclosed herein.

In yet another aspect, the present disclosure refers to the use of a peptide as disclosed herein in the manufacture of a medicament for preventing or treating a metabolic syndrome.

In yet another example, the present disclosure refers to a pharmaceutical composition for preventing or treating metabolic syndrome comprising a peptide as disclosed herein.

In a specific example, the peptide is an analog of native glucagon with a variation selected from the group consisting of substitution, addition, deletion, modification, and a combination thereof, on at least one amino acid of the native glucagon sequence.

In another specific example, the amino acid sequence with addition is derived from a native GLP-1 amino acid sequence, a native GIP amino acid sequence, or a native exendin-4 amino acid sequence.

In still another specific example, the peptide is an isolated peptide including an amino acid sequence represented by General Formula 1 below:

Xaa1-Xaa2-Xaa3-Gly-Thr-Phe-Xaa7-Ser-Asp-Xaa10-Ser-Xaa12-Xaa13-Xaa14-Xaa15-Xaa16-Xaa17-Xaa18-Xaa19-Xaa20-Xaa21-Phe-Xaa23-Xaa24-Trp-Leu-Xaa27-Xaa28-Xaa29-Xaa30-R1 (General Formula 1, SEQ ID NO: 103)

wherein, in General Formula 1,

Xaa1 is histidine (His, H), 4-imidazoacetyl (CA), or tyrosine (Tyr, Y);

Xaa2 is glycine (Gly, G), α -methyl-glutamic acid, or Aib (aminoisobutyric acid);
Xaa3 is glutamic acid (Glu, E) or glutamine (Gln, Q);
Xaa7 is threonine (Thr, T) or isoleucine (Ile, I);
Xaa10 is leucine (Leu, L), tyrosine (Tyr, Y), lysine (Lys, K), cysteine (Cys, C), or
valine (Val, V);
Xaa12 is lysine (Lys, K), serine (Ser, S), or isoleucine (Ile, I);
Xaa13 is glutamine (Gln, Q), tyrosine (Tyr, Y), alanine (Ala, A), or cysteine (Cys, C);
Xaa14 is leucine (Leu, L), methionine (Met, M), or tyrosine (Tyr, Y);

Xaa15 is cysteine (Cys, C), aspartic acid (Asp, D), glutamic acid (Glu, E), or leucine (Leu, L);

Xaa16 is glycine (Gly, G), glutamic acid (Glu, E), or serine (Ser, S);

Xaa17 is glutamine (Gln, Q), arginine (Arg, R), isoleucine (Ile, I), glutamic acid (Glu, E), cysteine (Cys, C), or lysine (Lys, K);

Xaa18 is alanine (Ala, A), glutamine (Gln, Q), arginine (Arg, R), or histidine (His, H);

Xaa19 is alanine (Ala, A), glutamine (Gln, Q), cysteine (Cys, C), or valine (Val, V);

Xaa20 is lysine (Lys, K), glutamine (Gln, Q), or arginine (Arg, R);

Xaa21 is glutamic acid (Glu, E), glutamine (Gln, Q), leucine (Leu, L), cysteine (Cys, C), or aspartic acid (Asp, D);

Xaa23 is isoleucine (Ile, I) or valine (Val, V);

Xaa24 is alanine (Ala, A), glutamine (Gln, Q), cysteine (Cys, C), asparagine (Asn, N), aspartic acid (Asp, D), or glutamic acid (Glu, E);

Xaa27 is valine (Val, V), leucine (Leu, L), lysine (Lys, K), or methionine (Met, M);

Xaa28 is cysteine (Cys, C), lysine (Lys, K), alanine (Ala, A), asparagine (Asn, N), or aspartic acid (Asp, D);

Xaa29 is cysteine (Cys, C), glycine (Gly, G), glutamine (Gln, Q), threonine (Thr, T), glutamic acid (Glu, E), or histidine (His, H);

Xaa30 is cysteine (Cys, C), glycine (Gly, G), lysine (Lys, K), or histidine (His, H), or is absent; and

R1 is cysteine (Cys, C), GKKNDWKHNIT (SEQ ID NO: 106), m-SSGAPPPS-n (SEQ ID NO: 107), or m-SSGQPPPS-n (SEQ ID NO: 108), or is absent;

wherein,

m is -Cys-, -Pro-, or -Gly-Pro-,

n is -Cys-, -Gly-, -Ser-, or -His-Gly-, or is absent.

In still another specific example,

in General Formula 1,

Xaa14 is leucine or methionine; and

Xaa15 is cysteine, aspartic acid, or leucine.

In still another specific example, in General Formula 1,

Xaa2 is glycine, α -methyl-glutamic acid, or Aib;

Xaa7 is threonine;

Xaa10 is tyrosine, cysteine, or valine;

Xaa12 is lysine or isoleucine;

Xaa13 is tyrosine, alanine, glutamine, or cysteine;

Xaa14 is leucine, cysteine, or methionine;

Xaa15 is cysteine, leucine, glutamic acid, or aspartic acid;

Xaa17 is glutamine, arginine, isoleucine, cysteine, glutamic acid, or lysine;

Xaa18 is alanine, glutamine, arginine, or histidine;

Xaa19 is alanine, glutamine, valine, or cysteine;

Xaa20 is lysine, arginine, or glutamine;

Xaa21 is glutamic acid, glutamine, leucine, cysteine, or aspartic acid;

Xaa23 is isoleucine or valine;

Xaa24 is cysteine, alanine, glutamine, asparagine, glutamic acid, or aspartic acid; and

Xaa27 is leucine or lysine.

In still another specific example, the peptide includes an amino acid sequence represented by General Formula 2 below:

Xaa1-Xaa2-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Xaa10-Ser-Lys-Xaa13-Xaa14-Xaa15-Xaa16-Xaa17-Xaa18-Xaa19-Xaa20-Xaa21-Phe-Xaa23-Xaa24-Trp-Leu-Leu-Xaa28-Xaa29-Xaa30-Xaa31-Ser-Ser-Gly-Gln-Pro-Pro-Pro-Ser-Xaa40 (General Formula 2, SEQ ID NO: 104)

In General Formula 2,

Xaa1 is 4-imidazoacetyl, histidine, or tyrosine;

Xaa2 is glycine, α -methyl-glutamic acid, or Aib;

Xaa10 is tyrosine or cysteine;

Xaa13 is alanine, glutamine, tyrosine, or cysteine;

Xaa14 is leucine, methionine, or tyrosine;

Xaa15 is aspartic acid, glutamic acid, or leucine;

Xaa16 is glycine, glutamic acid, or serine;

Xaa17 is glutamine, arginine, isoleucine, glutamic acid, cysteine, or lysine;
Xaa18 is alanine, glutamine, arginine, or histidine;
Xaa19 is alanine, glutamine, cysteine, or valine;
Xaa20 is lysine, glutamine, or arginine;
Xaa21 is cysteine, glutamic acid, glutamine, leucine, or aspartic acid;
Xaa23 is isoleucine or valine;
Xaa24 is cysteine, alanine, glutamine, asparagine, or glutamic acid;
Xaa28 is lysine, cysteine, asparagine, or aspartic acid;
Xaa29 is glycine, glutamine, cysteine, or histidine;
Xaa30 is cysteine, glycine, lysine, or histidine;
Xaa31 is proline or cysteine; and
Xaa40 is cysteine or is absent.

In still another specific example, in General Formula 1,

Xaa2 is glycine, α -methyl-glutamic acid, or Aib;
Xaa7 is threonine;
Xaa10 is tyrosine, cysteine, or valine;
Xaa12 is lysine or isoleucine;
Xaa13 is tyrosine, alanine, or cysteine;
Xaa14 is leucine or methionine;
Xaa15 is cysteine or aspartic acid;
Xaa17 is glutamine, arginine, isoleucine, cysteine, or lysine;
Xaa18 is alanine, arginine, or histidine;
Xaa19 is alanine, glutamine, or cysteine;
Xaa20 is lysine or glutamine;
Xaa21 is glutamic acid, cysteine, or aspartic acid;
Xaa23 is valine;
Xaa24 is alanine, glutamine, cysteine, asparagine, or aspartic acid; and
Xaa27 is leucine or lysine.

In still another specific example, in General Formula 2,

Xaa13 is alanine, tyrosine, or cysteine;

Xaa15 is aspartic acid or glutamic acid;
Xaa17 is glutamine, arginine, cysteine, or lysine;
Xaa18 is alanine, arginine, or histidine;
Xaa21 is cysteine, glutamic acid, glutamine, or aspartic acid;
Xaa23 is isoleucine or valine;
Xaa24 is cysteine, glutamine, or asparagine;
Xaa28 is cysteine, asparagine, or aspartic acid;
Xaa29 is glutamine, cysteine, or histidine; and
Xaa30 is cysteine, lysine, or histidine.

In still another specific example, in General Formula 1,

Xaa2 is α -methyl-glutamic acid or Aib;
Xaa7 is threonine;
Xaa10 is tyrosine or cysteine;
Xaa12 is lysine or isoleucine;
Xaa13 is tyrosine, alanine, or cysteine;
Xaa14 is leucine or methionine;
Xaa15 is cysteine or aspartic acid;
Xaa16 is glutamic acid;
Xaa17 is arginine, isoleucine, cysteine, or lysine;
Xaa18 is alanine, arginine, or histidine;
Xaa19 is alanine, glutamine, or cysteine;
Xaa20 is lysine or glutamine;
Xaa21 is glutamic acid or aspartic acid;
Xaa23 is valine;
Xaa24 is glutamine, asparagine, or aspartic acid;
Xaa27 is leucine; and
Xaa28 is cysteine, alanine, asparagine, or aspartic acid.

In still another specific example, in General Formula 1,

Xaa1 is histidine or 4-imidazoacetyl;
Xaa2 is α -methyl-glutamic acid or Aib;

Xaa3 is glutamine;
Xaa7 is threonine;
Xaa10 is tyrosine;
Xaa12 is isoleucine;
Xaa13 is alanine or cysteine;
Xaa14 is methionine;
Xaa15 is aspartic acid;
Xaa16 is glutamic acid;
Xaa17 is isoleucine or lysine;
Xaa18 is alanine or histidine;
Xaa19 is glutamine or cysteine;
Xaa20 is lysine;
Xaa21 is aspartic acid;
Xaa23 is valine;
Xaa24 is asparagine;
Xaa27 is leucine;
Xaa28 is alanine or asparagine;
Xaa29 is glutamine or threonine; and
Xaa30 is cysteine, or lysine, or is absent.

In still another specific example,
in General Formula 1,

Xaa2 is glycine, α -methyl-glutamic acid, or Aib;
Xaa3 is glutamine;
Xaa7 is threonine;
Xaa10 is tyrosine, cysteine, or valine;
Xaa12 is lysine;
Xaa13 is tyrosine;
Xaa14 is leucine;
Xaa15 is aspartic acid;
Xaa16 is glycine, glutamic acid, or serine;
Xaa17 is glutamine, arginine, cysteine, or lysine;

Xaa18 is alanine, arginine, or histidine;
Xaa19 is alanine or glutamine;
Xaa20 is lysine or glutamine;
Xaa21 is glutamic acid, cysteine, or aspartic acid;
Xaa23 is valine;
Xaa24 is alanine, glutamine, or cysteine;
Xaa27 is leucine or lysine; and
Xaa29 is glycine, glutamine, threonine, or histidine.

In still another specific example, the peptide is a peptide including an amino acid sequence represented by General Formula 3 below:

Xaa1-Xaa2-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Xaa13-Leu-Asp-Glu-Xaa17-Xaa18-Xaa19-Lys-Xaa21-Phe-Val-Xaa24-Trp-Leu-Leu-Xaa28-Xaa29-Xaa30-Xaa31-Ser-Ser-Gly-Gln-Pro-Pro-Pro-Ser-Xaa40 (General Formula 3, SEQ ID NO: 105)

In General Formula 3,
Xaa1 is histidine or tyrosine;
Xaa2 is α -methyl-glutamic acid or Aib;
Xaa13 is alanine, tyrosine, or cysteine;
Xaa17 is arginine, cysteine, or lysine;
Xaa18 is alanine or arginine;
Xaa19 is alanine or cysteine;
Xaa21 is glutamic acid or aspartic acid;
Xaa24 is glutamine or asparagine;
Xaa28 is cysteine or aspartic acid;
Xaa29 is cysteine, histidine, or glutamine;
Xaa30 is cysteine or histidine;
Xaa31 is proline or cysteine; and
Xaa40 is cysteine or is absent.

In still another specific example, R1 is cysteine, CSSGQPPPS (SEQ ID NO: 109),

GPSSGAPPPS (SEQ ID NO: 110), GPSSGAPPPSC (SEQ ID NO: 111), PSSGAPPPS (SEQ ID NO: 112), PSSGAPPPSG (SEQ ID NO: 113), PSSGAPPPSHG (SEQ ID NO: 114), PSSGAPPPSS (SEQ ID NO: 115), PSSGQPPPS (SEQ ID NO: 116), or PSSGQPPPS (SEQ ID NO: 117), or is absent.

In still another specific example, in General Formulas 1 to 3, the 16th amino acid and the 20th amino acid from the N-terminus together form a ring.

In still another specific example, the peptide includes an amino acid sequence selected from the group consisting of SEQ ID NOS: 1 to 102.

In still another specific example, the peptide has at least one of the following activities of i) to iii):

- i) activation of a GLP-1 receptor;
- ii) activation of a glucagon receptor; and
- iii) activation of a GIP receptor.

In still another specific example, the peptide has an increased *in vivo* half-life compared to that of any of native GLP-1, native glucagon, and native GIP.

In still another specific example, the C-terminus of the peptide is amidated.

Another example of the present disclosure provides a polynucleotide encoding the isolated peptide, a recombinant expression vector including the polynucleotide, and a transformant including the polynucleotide or the recombinant expression vector.

Still another example of the present disclosure provides a method for preparing the isolated peptide.

Still another example of the present disclosure provides a composition including the isolated peptide.

In a specific example, the composition is a pharmaceutical composition.

In another specific example, the composition is for preventing or treating metabolic syndrome.

In still another specific example, the metabolic syndrome includes impaired glucose tolerance, hypercholesterolemia, dyslipidemia, obesity, diabetes, hypertension, arteriosclerosis due to dyslipidemia, atherosclerosis, arteriosclerosis, or coronary heart disease.

Still another object of the present disclosure is to provide a method for treating a target disease, which includes administering the isolated peptide or a composition containing the isolated peptide to a subject in need thereof.

Still another object of the present disclosure is to provide a use of the isolated peptide or composition for use in the preparation of a medicament.

[Advantageous Effects of the Invention]

The peptide according to the present invention has activities to a glucagon receptor, a glucagon-like peptide-1 (GLP-1) receptor, and a glucose-dependent insulintropic polypeptide (GIP) receptor, and thus can be applied for the treatment of metabolic syndrome.

[DETAILED DESCRIPTION OF THE INVENTION]

Hereinafter, the present invention will be described in more detail.

Meanwhile, each of the explanations and exemplary embodiments disclosed herein can be applied to other explanations and exemplary embodiments. That is, all of the combinations of various factors disclosed herein belong to the scope of the present invention. Furthermore, the scope of the present invention should not be limited by the specific disclosure provided hereinbelow.

Over the entire specification of the present invention, not only the conventional one-letter and three-letter codes for naturally occurring amino acids, but also those three-letter codes generally allowed for other amino acids, such as α -aminoisobutyric acid (Aib), Sar (N-methylglycine), and α -methyl-glutamic acid, are used.

Additionally, the amino acids mentioned herein are abbreviated according to the nomenclature rules of IUPAC-IUB as follows:

alanine (Ala, A)	arginine (Arg, R)
asparagine (Asn, N)	aspartic acid (Asp, D)
cysteine (Cys, C)	glutamic acid (Glu, E)
glutamine (Gln, Q)	glycine (Gly, G)
histidine (His, H)	isoleucine (Ile, I)
leucine (Leu, L)	lysine (Lys, K)
methionine (Met, M)	phenylalanine (Phe, F)
proline (Pro, P)	serine (Ser, S)
threonine (Thr, T)	tryptophan (Trp, W)
tyrosine (Tyr, Y)	valine (Val, V)

An aspect of the present invention provides an isolated peptide which has activities to a glucagon receptor, a glucagon-like peptide-1 (GLP-1) receptor, and a glucose-dependent insulintropic polypeptide (GIP) receptor.

In the present invention, the isolated peptide having activities to all of the glucagon receptor, a glucagon-like peptide-1 (GLP-1) receptor, and a glucose-dependent insulintropic polypeptide (GIP) receptor can be used interchangeably with a triple agonist.

The triple agonist may include various materials (*e.g.*, various peptides) which have a significant level of activities to glucagon, GLP-1, and GIP receptors.

The triple agonist having a significant level of activities to glucagon, GLP-1, and GIP receptors may exhibit *in vitro* activities of 0.1% or higher, 1% or higher, 2% or higher, 3% or higher, 4% or higher, 5% or higher, 6% or higher, 7% or higher, 8% or higher, 9% or higher, 10% or higher, 20% or higher, 30% or higher, 40% or higher, 50% or higher, 60% or higher, 70% or higher, 80% or higher, 90% or higher, and 100% or higher, to one or more receptors, specifically two or more receptors, and more specifically all three of the receptors among the glucagon, GLP-1, and GIP receptors, compared to native ligands of the corresponding receptors (native glucagon, native GLP-1, and native GIP), but is not particularly limited thereto.

The method for measuring the *in vitro* activities of the triple agonist may refer to Example 2 of the present invention, but is not particularly limited thereto.

Meanwhile, the triple agonist is characterized by having one or more of the following activities of i) to iii), specifically a significant activity(-ies) thereof:

i) activation of a GLP-1 receptor; ii) activation of a glucagon receptor; and iii) activation of a GIP receptor.

In particular, the activation of receptors may include, for example, those cases where the *in vitro* activities are 0.1% or higher, 1% or higher, 2% or higher, 3% or higher, 4% or higher, 5% or higher, 6% or higher, 7% or higher, 8% or higher, 9% or higher, 10% or higher, 20% or higher, 30% or higher, 40% or higher, 50% or higher, 60% or higher, 70% or higher, 80% or higher, 90% or higher, and 100% or higher, compared to native ligands of the corresponding receptors, but the activation is not limited thereto.

Additionally, the triple agonist may be one which has an increased *in vivo* half-life relative to any one of native GLP-1, native glucagon, and native GIP, but is not particularly limited thereto.

The above glucagon analog may be one which is non-naturally occurring, but is not particularly limited thereto.

Specifically, the isolated peptide may be an analog of native glucagon, but is not particularly limited thereto.

The native glucagon analog according to the present invention may include peptides which have at least one difference in amino acid sequence compared to that of native glucagon; peptides which were modified via modification of the native glucagon sequence; and mimetics of the native glucagon.

Meanwhile, native glucagon may have the following amino acid sequence, but is not particularly limited thereto:

His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr (SEQ ID NO: 118)

Specifically, the isolated peptide may be an analog of native glucagon with a variation selected from the group consisting of substitution, addition, deletion, modification, and a combination thereof, on at least one amino acid of the native glucagon sequence, but is not particularly limited thereto.

Additionally, the substitution of the amino acid may include both a substitution to an amino acid and a substitution to a non-native compound.

Additionally, the addition may be performed at the N-terminus and/or C-terminus of a peptide. Meanwhile, the length of the amino acid for addition is not particularly limited, but 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, and 11 or more amino acids may be added, and in a broad sense, the addition may include the addition of a polypeptide, but is not particularly limited thereto.

More specifically, the glucagon analog may be those where 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, or 20 amino acids selected from the group consisting of amino acids at positions 1, 2, 3, 7, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 23, 24, 27, 28, and 29 in the amino acid sequence of native glucagon are substituted with other amino acids, and in addition, may be those where 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, or 11 or more amino acids are independently or additionally added to the C-terminus thereof, but is not particularly limited thereto.

Even more specifically, the glucagon analog may be those where 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, or 19 amino acids selected from the group consisting of amino acids at positions 1, 2, 3, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 23, 24, 27, 28, and 29 in the amino acid sequence of native glucagon are substituted with other amino acids, and in addition, may be those where 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, or 11 or more amino acids are independently or additionally added to the C-terminus thereof, but is not particularly limited thereto.

Even more specifically, the glucagon analog may be those where 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or

more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, or 17 amino acids selected from the group consisting of amino acids at positions 1, 2, 3, 10, 13, 14, 15, 16, 17, 18, 19, 20, 21, 23, 24, 28, and 29 in the amino acid sequence of native glucagon are substituted with other amino acids, and in addition, may be those where 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, or 11 or more amino acids are independently or additionally added to the C-terminus thereof, but is not particularly limited thereto.

Even more specifically, the glucagon analog may be those where 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, or 14 amino acids selected from the group consisting of amino acids at positions 1, 2, 13, 16, 17, 18, 19, 20, 21, 23, 24, 27, 28, and 29 in the amino acid sequence of native glucagon are substituted with other amino acids, and in addition, may be those where 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, or 11 or more amino acids are independently or additionally added to the C-terminus thereof, but is not particularly limited thereto.

The amino acids to be introduced into the above native glucagon may be selected from the group consisting of tyrosine, α -methyl-glutamic acid, Aib, methionine, glutamic acid, histidine, lysine, leucine, isoleucine, glutamine, valine, glycine, alanine, cysteine, serine, alanine, aspartic acid, and arginine, but are not particularly limited thereto.

For example, the amino acid sequence(s) to be added may be at least one amino acid sequence derived from a native GLP-1, native GIP, or native exendin-4 amino acid sequence.

The glucagon analog or triple agonist may include an intramolecular bridge (*e.g.*, a covalent crosslinking or non-covalent crosslinking), and specifically, is in a form including a ring, for example, is in a form where a ring is formed between the 16th amino acid and the 20th amino acid of the glucagon analog or the triple agonist, but is not particularly limited thereto.

The non-limiting example of the ring may include a lactam bridge (or a lactam ring).

Additionally, the glucagon analog or triple agonist includes all of those which are modified to include a ring, or include an amino acid capable of forming a ring in a target position.

For example, the glucagon analog or triple agonist may be one where the amino acid pair of the 16th and 20th amino acids are substituted with glutamic acid or lysine, which can form a ring, respectively, but the glucagon analog or triple agonist are not limited thereto.

The ring may be formed between amino acid side chains within the glucagon analog or triple agonist; for example, they may be in the form of a lactam ring between a side chain of lysine and a side chain of glutamic acid, but the ring is not particularly limited thereto.

Examples of the glucagon analog prepared by a combination of these methods may include peptides, whose amino acid sequences differ from that of native glucagon in at least one amino acid, and in which the α -carbon in the N-terminus thereof is removed, while having activities to a glucagon receptor, a GLP-1 receptor, and a GIP receptor, *etc.*, but are not limited thereto, and analogs of native glucagon applicable to the present invention can be prepared by combining various methods for the preparation of analogs.

Additionally, with respect to the triple agonist of the present invention, a part of the amino acids may be substituted with other amino acids or non-natural compounds to avoid the recognition by peptidase for increasing the *in vivo* half life of the triple agonist, but the triple agonist is not particularly limited thereto.

Specifically, the peptide may be a peptide where the *in vivo* half life was increased by avoiding the recognition by the peptidase via substitution of the 2nd amino acid sequence among the amino acid sequences of the triple agonist, but any substitution or modification of amino acids to avoid the recognition by *in vivo* peptidase is included without limitation.

Additionally, such modification for preparing analogs of native glucagon may include all of the modifications using *L*-type or *D*-type amino acids and/or non-natural amino acids; and/or a modification of native sequence, for example, a modification of a side chain functional group, an intramolecular covalent bonding (*e.g.*, a ring formation between side chains), methylation, acylation, ubiquitination, phosphorylation, aminohexanation, biotinylation, *etc.*

Additionally, the modification may also include all of those where one or more amino acids are added to the amino and/or carboxy terminus of native glucagon.

During the substitution or addition of amino acids, not only the 20 amino acids commonly found in human proteins, but also atypical or non-naturally occurring amino acids may be used. Commercial sources of atypical amino acids may include Sigma-Aldrich, ChemPep Inc., and Genzyme Pharmaceuticals. The peptides including these amino acids

and typical peptide sequences may be synthesized and purchased from commercial suppliers, *e.g.*, American Peptide Company, Bachem (USA), or Anygen (Korea).

Amino acid derivatives may be obtained in the same manner, and as one such example, 4-imidazoacetic acid may be used.

Additionally, the peptide according to the present invention may be in the form of a variant where the amino and/or carboxy terminus, *etc.* of the peptide is chemically modified or protected by organic groups, or amino acids may be added to the terminus of the peptide, for its protection from proteases *in vivo* while increasing its stability.

In particular, in the case of a chemically-synthesized peptide, its N- and C-termini are electrically charged and thus the N- and C-termini of the peptide may be acetylated and/or amidated, but the peptide is not particularly limited thereto.

Additionally, the peptide according to the present invention may include all of those in the form of the peptide itself, a salt thereof (*e.g.*, a pharmaceutically acceptable salt thereof), or a solvate thereof. Additionally, the peptide may be in any pharmaceutically acceptable form.

The kind of the salt is not particularly limited. However, the salt is preferably one that is safe and effective to a subject, *e.g.*, a mammal, but is not particularly limited thereto.

The term “pharmaceutically acceptable” refers to a material which can be effectively used for the intended use within the scope of pharmaco-medical decision without inducing excessive toxicity, irritation, allergic responses, *etc.*

As used herein, the term “pharmaceutically acceptable salt” refers to a salt derived from pharmaceutically acceptable inorganic acids, organic acids, or bases. Examples of the suitable salts may include hydrochloric acid, bromic acid, sulfuric acid, nitric acid, perchloric acid, fumaric acid, maleic acid, phosphoric acid, glycolic acid, lactic acid, salicylic acid, succinic acid, toluene-*p*-sulfonic acid, tartaric acid, acetic acid, citric acid, methanesulfonic acid, formic acid, benzoic acid, malonic acid, naphthalene-2-sulfonic acid, benzenesulfonic acid, *etc.* Examples of the salts derived from suitable bases may include alkali metals such as sodium, potassium, *etc.*; alkali earth metals such as magnesium; ammonium, *etc.*

As used herein, the term “solvate” refers to a complex formed between the peptide according to the present invention or a salt thereof and a solvent molecule.

In a specific embodiment, the peptide may be an isolated peptide which includes an amino acid sequence represented by General Formula 1 below.

Xaa1-Xaa2-Xaa3-Gly-Thr-Phe-Xaa7-Ser-Asp-Xaa10-Ser-Xaa12-Xaa13-Xaa14-Xaa15-Xaa16-Xaa17-Xaa18-Xaa19-Xaa20-Xaa21-Phe-Xaa23-Xaa24-Trp-Leu-Xaa27-Xaa28-Xaa29-Xaa30-R1 (General Formula 1, SEQ ID NO: 103)

In General Formula 1 above,

Xaa1 is histidine (His, H), 4-imidazoacetyl (CA), or tyrosine (Tyr, Y);

Xaa2 is glycine (Gly, G), α -methyl-glutamic acid, or Aib (aminoisobutyric acid);

Xaa3 is glutamic acid (Glu, E) or glutamine (Gln, Q);

Xaa7 is threonine (Thr, T) or isoleucine (Ile, I);

Xaa10 is leucine (Leu, L), tyrosine (Tyr, Y), lysine (Lys, K), cysteine (Cys, C), or valine (Val, V);

Xaa12 is lysine (Lys, K), serine (Ser, S), or isoleucine (Ile, I);

Xaa13 is glutamine (Gln, Q), tyrosine (Tyr, Y), alanine (Ala, A), or cysteine (Cys, C);

Xaa14 is leucine (Leu, L), methionine (Met, M), or tyrosine (Tyr, Y);

Xaa15 is cysteine (Cys, C), aspartic acid (Asp, D), glutamic acid (Glu, E), or leucine (Leu, L);

Xaa16 is glycine (Gly, G), glutamic acid (Glu, E), or serine (Ser, S);

Xaa17 is glutamine (Gln, Q), arginine (Arg, R), isoleucine (Ile, I), glutamic acid (Glu, E), cysteine (Cys, C), or lysine (Lys, K);

Xaa18 is alanine (Ala, A), glutamine (Gln, Q), arginine (Arg, R), or histidine (His, H);

Xaa19 is alanine (Ala, A), glutamine (Gln, Q), cysteine (Cys, C), or valine (Val, V);

Xaa20 is lysine (Lys, K), glutamine (Gln, Q), or arginine (Arg, R);

Xaa21 is glutamic acid (Glu, E), glutamine (Gln, Q), leucine (Leu, L), cysteine (Cys, C), or aspartic acid (Asp, D);

Xaa23 is isoleucine (Ile, I) or valine (Val, V);

Xaa24 is alanine (Ala, A), glutamine (Gln, Q), cysteine (Cys, C), asparagine (Asn, N), aspartic acid (Asp, D), or glutamic acid (Glu, E);

Xaa27 is valine (Val, V), leucine (Leu, L), lysine (Lys, K), or methionine (Met, M);

Xaa28 is cysteine (Cys, C), lysine (Lys, K), alanine (Ala, A), asparagine (Asn, N), or aspartic acid (Asp, D);

Xaa29 is cysteine (Cys, C), glycine (Gly, G), glutamine (Gln, Q), threonine (Thr, T), glutamic acid (Glu, E), or histidine (His, H);

Xaa30 is cysteine (Cys, C), glycine (Gly, G), lysine (Lys, K), or histidine (His, H), or is absent;

R1 is cysteine (Cys, C), GKKNDWKHNIT (SEQ ID NO: 106), m-SSGAPPPS-n (SEQ ID NO: 107), or m-SSGQPPPS-n (SEQ ID NO: 108), or is absent;

wherein,

m is -Cys-, -Pro-, or -Gly-Pro-;

n is -Cys-, -Gly-, -Ser-, or -His-Gly-, or is absent.

For example, the triple agonist may be one which includes an amino acid sequence selected from the group consisting of SEQ ID NOS: 1 to 102; and one which (essentially) consists of an amino acid sequence selected from the group consisting of SEQ ID NOS: 1 to 102, but is not limited thereto.

Additionally, although described as “a peptide consisting of a particular SEQ ID NO” in the present invention, it does not exclude a mutation that may occur by the addition of a meaningless sequence upstream or downstream of the amino acid sequence of the corresponding SEQ ID NO, or a mutation that may occur naturally, or a silent mutation thereof, as long as the peptide has an activity the same as or corresponding to that of the peptide which consists of an amino acid sequence of the corresponding SEQ ID NO, and even when the sequence addition or mutation is present, it obviously belongs to the scope of the present invention.

The above may be applicable in other specific embodiments or aspects of the present invention, but is not limited thereto.

Specifically, in General Formula above, Xaa14 may be leucine or methionine, and Xaa15 may be cysteine, aspartic acid, or leucine.

Examples of the peptide may include a peptide which includes an amino acid sequence selected from the group consisting of SEQ ID NOS: 1 to 12, 14 to 17, and 21 to 102 or a peptide which (essentially) consists of the same, but are not particularly limited thereto.

The peptide may significantly activate at least one of the glucagon receptor, GLP-1

receptor, and GIP receptor, but is not particularly limited thereto. Specifically, the peptide may be one which significantly activates the GLP-1 receptor, or additionally the glucagon receptor and/or GIP receptor, but is not particularly limited thereto.

Even more specifically, the peptide may be:

in General Formula 1 above,

Xaa2 is glycine, α -methyl-glutamic acid, or Aib;

Xaa7 is threonine;

Xaa10 is tyrosine, cysteine, or valine;

Xaa12 is lysine or isoleucine;

Xaa13 is tyrosine, alanine, glutamine, or cysteine;

Xaa14 is leucine, cysteine, or methionine;

Xaa15 is cysteine, leucine, glutamic acid, or aspartic acid;

Xaa17 is glutamine, arginine, isoleucine, cysteine, glutamic acid, or lysine;

Xaa18 is alanine, glutamine, arginine, or histidine;

Xaa19 is alanine, glutamine, valine, or cysteine;

Xaa20 is lysine, arginine, or glutamine;

Xaa21 is glutamic acid, glutamine, leucine, cysteine, or aspartic acid;

Xaa23 is isoleucine or valine;

Xaa24 is cysteine, alanine, glutamine, asparagine, glutamic acid, or aspartic acid; and

Xaa27 is leucine or lysine, but is not particularly limited thereto.

Even more specifically,

in General Formula 1 above,

Xaa2 is glycine, α -methyl-glutamic acid, or Aib;

Xaa7 is threonine;

Xaa10 is tyrosine, cysteine, or valine;

Xaa12 is lysine or isoleucine;

Xaa13 is tyrosine, alanine, or cysteine;

Xaa14 is leucine or methionine;

Xaa15 is cysteine or aspartic acid;

Xaa17 is glutamine, arginine, isoleucine, cysteine, or lysine;

Xaa18 is alanine, arginine, or histidine;
Xaa19 is alanine, glutamine, or cysteine;
Xaa20 is lysine or glutamine;
Xaa21 is glutamic acid, cysteine, or aspartic acid;
Xaa23 is valine;
Xaa24 is alanine, glutamine, cysteine, asparagine, or aspartic acid; and
Xaa27 is leucine or lysine, but is not particularly limited thereto.

Even more specifically,
in General Formula 1 above,
Xaa2 is α -methyl-glutamic acid or Aib;
Xaa7 is threonine;
Xaa10 is tyrosine or cysteine;
Xaa12 is lysine or isoleucine;
Xaa13 is tyrosine, alanine, or cysteine;
Xaa14 is leucine or methionine;
Xaa15 is cysteine or aspartic acid;
Xaa16 is glutamic acid;
Xaa17 is arginine, isoleucine, cysteine, or lysine;
Xaa18 is alanine, arginine, or histidine;
Xaa19 is alanine, glutamine, or cysteine;
Xaa20 is lysine or glutamine;
Xaa21 is glutamic acid or aspartic acid;
Xaa23 is valine;
Xaa24 is glutamine, asparagine, or aspartic acid;
Xaa27 is leucine; and
Xaa28 is cysteine, alanine, asparagine, or aspartic acid.

Specifically,
in General Formula 1 above,
Xaa1 is histidine or 4-imidazoacetyl;
Xaa2 is α -methyl-glutamic acid or Aib;

Xaa3 is glutamine;
Xaa7 is threonine;
Xaa10 is tyrosine;
Xaa12 is isoleucine;
Xaa13 is alanine or cysteine;
Xaa14 is methionine;
Xaa15 is aspartic acid;
Xaa16 is glutamic acid;
Xaa17 is isoleucine or lysine;
Xaa18 is alanine or histidine;
Xaa19 is glutamine or cysteine;
Xaa20 is lysine;
Xaa21 is aspartic acid;
Xaa23 is valine;
Xaa24 is asparagine;
Xaa27 is leucine;
Xaa28 is alanine or asparagine;
Xaa29 is glutamine or threonine; and
Xaa30 is cysteine or lysine, or is absent.

More specifically,
in General Formula 1 above,
Xaa2 is glycine, α -methyl-glutamic acid, or Aib;
Xaa3 is glutamine;
Xaa7 is threonine;
Xaa10 is tyrosine, cysteine, or valine;
Xaa12 is lysine;
Xaa13 is tyrosine;
Xaa14 is leucine;
Xaa15 is aspartic acid;
Xaa16 is glycine, glutamic acid, or serine;
Xaa17 is glutamine, arginine, cysteine, or lysine;

Xaa18 is alanine, arginine, or histidine;
Xaa19 is alanine or glutamine;
Xaa20 is lysine or glutamine;
Xaa21 is glutamic acid, cysteine, or aspartic acid;
Xaa23 is valine;
Xaa24 is alanine, glutamine, or cysteine;
Xaa27 is leucine or lysine; and
Xaa28 is glycine, glutamine, threonine, or histidine;
but is not particularly limited thereto.

These peptides may correspond to a case where the peptide has significant activation levels on both the GLP-1 receptor and glucagon receptor, or higher activation levels compared to that on the GIP receptor; a case where the peptide has significant activation levels on all of the GLP-1 receptor, glucagon receptor, and GIP receptor; or a case where the peptide has significant activation levels on both the GLP-1 receptor and GIP receptor and higher activation levels compared to that on the glucagon receptor; but are not particularly limited thereto.

When the peptide has significant activation levels on both the GLP-1 receptor and GIP receptor, and also higher activation levels compared to that on the glucagon receptor, it is possible to provide a peptide with more improved capability of controlling blood glucose levels along with the effect of reducing body weight, whereas when the peptide has significant activation levels on all of the GLP-1 receptor, glucagon receptor, and GIP receptor, there is an advantage in that the effect of reducing body weight can be maximized, but the effects are not particularly limited thereto.

Examples of the peptide may include a peptide which includes an amino acid sequence selected from the group consisting of SEQ ID NOS: 8, 9, 21 to 37, 39, 42, 43, 49 to 61, 64 to 83, 85, 86, 88, 89, 91 to 93, and 95 to 102; or a peptide which (essentially) consists of the same, but are not particularly limited thereto.

In a specific embodiment, the peptide may include an amino acid sequence represented by General Formula 2 below.

Xaa1-Xaa2-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Xaa10-Ser-Lys-Xaa13-Xaa14-Xaa15-Xaa16-Xaa17-Xaa18-Xaa19-Xaa20-Xaa21-Phe-Xaa23-Xaa24-Trp-Leu-Leu-Xaa28-Xaa29-Xaa30-Xaa31-Ser-Ser-Gly-Gln-Pro-Pro-Pro-Ser-Xaa40 (General Formula 2, SEQ ID NO: 104)

In General Formula 2 above,

Xaa1 is 4-imidazoacetyl, histidine, or tyrosine;

Xaa2 is glycine, α -methyl-glutamic acid, or Aib;

Xaa10 is tyrosine or cysteine;

Xaa13 is alanine, glutamine, tyrosine, or cysteine;

Xaa14 is leucine, methionine, or tyrosine;

Xaa15 is aspartic acid, glutamic acid, or leucine;

Xaa16 is glycine, glutamic acid, or serine;

Xaa17 is glutamine, arginine, isoleucine, glutamic acid, cysteine, or lysine;

Xaa18 is alanine, glutamine, arginine, or histidine;

Xaa19 is alanine, glutamine, cysteine, or valine;

Xaa20 is lysine, glutamine, or arginine;

Xaa21 is cysteine, glutamic acid, glutamine, leucine, or aspartic acid;

Xaa23 is isoleucine or valine;

Xaa24 is cysteine, alanine, glutamine, asparagine, or glutamic acid;

Xaa28 is lysine, cysteine, asparagine, or aspartic acid;

Xaa29 is glycine, glutamine, cysteine, or histidine;

Xaa30 is cysteine, glycine, lysine, or histidine;

Xaa31 is proline or cysteine; and

Xaa40 is cysteine or is absent.

More specifically, in General Formula 2 above,

Xaa13 is alanine, tyrosine, or cysteine;

Xaa15 is aspartic acid or glutamic acid;

Xaa17 is glutamine, arginine, cysteine, or lysine;

Xaa18 is alanine, arginine, or histidine;

Xaa21 is cysteine, glutamic acid, glutamine, or aspartic acid;

Xaa23 is isoleucine or valine;
Xaa24 is cysteine, glutamine, or asparagine;
Xaa28 is cysteine, asparagine, or aspartic acid;
Xaa29 is glutamine, cysteine, or histidine; and
Xaa30 is cysteine, lysine, or histidine.

Examples of the peptide may include a peptide which includes an amino acid sequence selected from the group consisting of SEQ ID NOS: 21, 22, 42, 43, 50, 64 to 77, and 95 to 102; more specifically, a peptide which includes an amino acid sequence selected from the group consisting of SEQ ID NOS: 21, 22, 42, 43, 50, 64 to 77, and 96 to 102; or a peptide which (essentially) consists of the same, but are not particularly limited thereto.

In a specific embodiment, the peptide may include an amino acid sequence represented by General Formula 3 below.

Xaa1-Xaa2-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Xaa13-Leu-Asp-Glu-Xaa17-Xaa18-Xaa19-Lys-Xaa21-Phe-Val-Xaa24-Trp-Leu-Leu-Xaa28-Xaa29-Xaa30-Xaa31-Ser-Ser-Gly-Gln-Pro-Pro-Pro-Ser-Xaa40 (General Formula 3, SEQ ID NO: 105),

In General Formula 3 above,
Xaa1 is histidine or tyrosine;
Xaa2 is α -methyl-glutamic acid or Aib;
Xaa13 is alanine, tyrosine or cysteine;
Xaa17 is arginine, cysteine, or lysine;
Xaa18 is alanine or arginine;
Xaa19 is alanine or cysteine;
Xaa21 is glutamic acid or aspartic acid;
Xaa24 is glutamine or asparagine,
Xaa28 is cysteine or aspartic acid;
Xaa29 is cysteine, histidine, or glutamine;
Xaa30 is cysteine or histidine;
Xaa31 is proline or cysteine; and

Xaa40 is cysteine or is absent.

Examples of the peptide may include a peptide which includes an amino acid sequence selected from the group consisting of SEQ ID NOS: 21, 22, 42, 43, 50, 64 to 71, 75 to 77, and 96 to 102; or a peptide which (essentially) consists of the same, but are not particularly limited thereto.

Additionally, in General Formula 1 above, R1 may be cysteine, GKKNDWKHNIT (SEQ ID NO: 106), CSSGQPPPS (SEQ ID NO: 109), GPSSGAPPPS (SEQ ID NO: 110), GPSSGAPPPSC (SEQ ID NO: 111), PSSGAPPPS (SEQ ID NO: 112), PSSGAPPPSG (SEQ ID NO: 113), PSSGAPPPSHG (SEQ ID NO: 114), PSSGAPPPSS (SEQ ID NO: 115), PSSGQPPPS (SEQ ID NO: 116), or PSSGQPPPS (SEQ ID NO: 117), or is absent, but is not particularly limited thereto.

Still another aspect of the present invention provides a polynucleotide encoding the isolated peptide, a recombinant expression vector including the polynucleotide, and a transformant including the polynucleotide or the recombinant expression vector.

The peptide is the same as explained above.

Additionally, the isolated polynucleotide encoding the peptide includes, within the scope of the present invention, a polynucleotide sequence having a sequence identity to the corresponding sequence of 75% or higher, specifically 85% or higher, more specifically 90% or higher, and even more specifically 95% or higher.

As used herein, the term “homology” indicates sequence similarity with a wild-type amino acid sequence or wild-type nucleic acid sequence, and the homology comparison may be done with the naked eye or using a commercially-available comparison program. Using a commercially available computer program, the homology between two or more sequences may be expressed as a percentage (%), and the homology (%) between adjacent sequences may be calculated.

As used herein, the term “recombinant vector” refers to a DNA construct in which the polynucleotide encoding the target peptide, e.g., the peptide, is operably linked to an appropriate regulatory sequence to enable the expression of the target peptide, e.g., the peptide, in a host cell.

The regulatory sequence includes a promoter capable of initiating transcription, any operator sequence for regulating the transcription, a sequence encoding an appropriate mRNA ribosome-binding domain, and a sequence for regulating the termination of transcription and translation. The recombinant vector, after being transformed into a suitable host cell, may be replicated or function irrespective of the host genome, or may be integrated into the host genome itself.

The recombinant vector used in the present invention may not be particularly limited as long as the vector is replicable in the host cell, and it may be constructed using any vector known in the art. Examples of the vector conventionally used may include natural or recombinant plasmids, cosmids, viruses, and bacteriophages. The vectors to be used in the present invention are not particularly limited but any expression vector known in the art may be used.

The recombinant vector is used for the transformation of a host cell for producing peptides of the present invention. Additionally, these transformed cells, as a part of the present invention, may be used for amplifying nucleic acid fragments and vectors, or they may be cultured cells or cell lines used in the recombinant production of peptides of the present invention.

As used herein, the term “transformation” refers to a process of introducing a recombinant vector including a polynucleotide encoding a target protein into a host cell, thereby enabling the expression of the protein encoded by the polynucleotide in the host cell. For the transformed polynucleotide, it does not matter whether it is inserted into the chromosome of a host cell and located thereon or located outside of the chromosome, as long as it can be expressed in the host cell, and both cases are included.

Additionally, the polynucleotide includes DNA and RNA which encode the target protein. The polynucleotide may be introduced in any form as long as it can be introduced into a host cell and expressed therein. For example, the polynucleotide may be introduced into a host cell in the form of an expression cassette, which is a gene construct including all of the essential elements required for self-expression. The expression cassette may conventionally include a promoter operably linked to the polynucleotide, a transcription termination signal, a ribosome-binding domain, and a translation termination signal. The expression cassette may be in the form of an expression vector capable of self-replication. Additionally, the polynucleotide may be introduced into a host cell as it is and operably

linked to a sequence essential for its expression in the host cell, but is not limited thereto.

Additionally, as used herein, the term “operably linked” refers to a functional connection between a promoter sequence, which initiates and mediates the transcription of the polynucleotide encoding the target peptide of the present invention, and the above gene sequence.

An appropriate host to be used in the present invention may not be particularly limited as long as it can express the polynucleotide of the present invention. Examples of the appropriate host may include bacteria belonging to the genus *Escherichia* such as *E. coli*; bacteria belonging to the genus *Bacillus* such as *Bacillus subtilis*; bacteria belonging to the genus *Pseudomonas* such as *Pseudomonas putida*; yeasts such as *Pichia pastoris*, *Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe*; insect cells such as *Spodoptera frugiperda* (Sf9), and animal cells such as CHO, COS, and BSC.

Still another aspect of the present invention provides a method for preparing the isolated peptide.

The peptide is the same as explained above.

Additionally, the peptide of the present invention may be synthesized by a method well-known in the art, according to its length, *e.g.*, by an automatic peptide synthesizer, and may be produced by genetic engineering technology.

Specifically, the peptide of the present invention may be prepared by a standard synthesis method, a recombinant expression system, or any other method known in the art. Accordingly, the peptide of the present invention may be synthesized by many methods including, for example, the methods described below:

(a) a method of synthesizing a peptide by a solid-phase or liquid-phase method stepwise or by fragment assembly, followed by isolation and purification of the final peptide product; or

(b) a method of expressing a nucleic acid construct encoding a peptide in a host cell and recovering the expression product from the host cell culture; or

(c) a method of performing an *in vitro* cell-free expression of a nucleic acid construct encoding a peptide and recovering the expression product therefrom; or

a method of obtaining peptide fragments by any combination of the methods (a), (b), and (c), obtaining the peptide by linking the peptide fragments, and then recovering the

peptide.

In a more specific example, a desired peptide may be produced by genetic manipulation, which includes preparing a fusion gene encoding a fusion protein, including a fusion partner and a peptide, transforming the resultant into a host cell, expressing the fusion protein, and cleaving the peptide from the fusion protein using a protease or compound followed by isolation. For this purpose, for example, a DNA sequence encoding the amino acid sequence that can be cleaved by a protease such as Factor Xa or enterokinase, CNBr, or a compound such as hydroxylamine, may be inserted between the fusion partner and a polynucleotide encoding a peptide.

Still another aspect of the present invention provides a composition containing the isolated peptide.

The peptide is the same as explained above.

Specifically, the composition may be a pharmaceutical composition, and more specifically, a pharmaceutical composition for preventing or treating metabolic syndrome.

As used herein, the term “prevention” refers to all activities that inhibit or delay metabolic syndrome by administering the above peptide or the composition, and the term “treatment” refers to all activities that improve or advantageously change the symptoms of metabolic syndrome by administering the above peptide or the composition.

As used herein, the term “administration” refers to the introduction of a particular substance into a subject by an appropriate method, and the administration route of the composition may be any conventional route that enables delivery of the composition to the target, for example, intraperitoneal administration, intravenous administration, intramuscular administration, subcutaneous administration, intradermal administration, oral administration, topical administration, intranasal administration, intrapulmonary administration, intrarectal administration, *etc.*, but is not limited thereto.

As used herein, the term “metabolic syndrome” refers to a symptom where various diseases that occur due to chronic metabolic disorder occur alone or in combination. In particular, examples of diseases that belong to metabolic syndrome may include impaired glucose tolerance, hypercholesterolemia, dyslipidemia, obesity, diabetes, hypertension, arteriosclerosis due to dyslipidemia, atherosclerosis, arteriosclerosis, and coronary heart disease, but are not limited thereto.

As used herein, the term “obesity” refers to a medical condition with excess body fat accumulation and people are generally defined to be obese when their body mass index (BMI; a value of body mass (kg) over body height squared (m)) is 25 or higher. Obesity is most commonly caused by energy imbalance due to excessive food intake compared to energy consumption over a long period of time. Obesity, being a metabolic disease that affects the entire body, increases the possibility of developing of diabetes and hyperlipidemia, increases the risk of the incidence of sexual dysfunction, arthritis, and cardiovascular disease, and is associated with cancer development in some cases.

The pharmaceutical composition of the present invention may further contain a pharmaceutically acceptable carrier, excipient, or diluent. The pharmaceutically acceptable carrier, excipient, or diluent may be non-naturally occurring.

As used herein, the term “pharmaceutically acceptable” refers to the properties of having a sufficient amount to exhibit a therapeutic effect and not causing adverse effects, and may be easily determined by a skilled person in the art based on the factors well-known in the medical field, such as the kind of disease, age, body weight, health status, sex, drug sensitivity of a patient, administration route, administration method, administration frequency, duration of treatment, a drug(s) to be mixed or administered simultaneously, *etc.*

The pharmaceutical composition of the present invention containing the peptide of the present invention may further contain a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier may include, for oral administration, a binder, a lubricant, a disintegrant, an excipient, a solubilizing agent, a dispersant, a stabilizing agent, a suspending agent, a coloring agent, a flavoring agent, *etc.*; for injections, a buffering agent, a preserving agent, an analgesic, a solubilizing agent, an isotonic agent, a stabilizing agent, *etc.*, which may be combined to be used; and for topical administrations, a base, an excipient, a lubricant, a preserving agent, *etc.*

The formulation type of the composition according to the present invention may be prepared variously by combining with a pharmaceutically acceptable carrier described above. For example, for oral administration, the composition may be formulated into tablets, troches, capsules, elixirs, suspensions, syrups, wafers, *etc.* For injections, the composition may be formulated into unit-dose ampoules or multi-dose containers. The composition may also be formulated into solutions, suspensions, tablets, pills, capsules, sustained-release formulations, *etc.*

Meanwhile, examples of suitable carriers, excipients, and diluents may include lactose, dextrose, sucrose, sorbitol, mannitol, xylitol, erythritol, maltitol, starch, acacia rubber, alginate, gelatin, calcium phosphate, calcium silicate, cellulose, methyl cellulose, microcrystalline cellulose, polyvinylpyrrolidone, water, methyl hydroxybenzoate, propyl hydroxybenzoate, talc, magnesium stearate, mineral oil, *etc.* Additionally, the composition may further contain a filler, an anti-coagulant, a lubricant, a humectant, a flavoring agent, a preservative, *etc.*

Additionally, the pharmaceutical composition of the present invention may be prepared in any formulation type selected from the group consisting of tablets, pills, powders, granules, capsules, suspensions, liquid medicine for internal use, emulsions, syrups, sterile aqueous solutions, non-aqueous solvents, lyophilized formulations, and suppositories.

Additionally, the composition may be formulated into a unit dosage form suitable for the patient's body, and is preferably formulated into a preparation useful for peptide drugs according to the typical method in the pharmaceutical field so as to be administered by an oral or parenteral route, such as through skin, intravenously, intramuscularly, intraarterially, intramedullarily, intrathecally, intraventricularly, pulmonarily, transdermally, subcutaneously, intraperitoneally, intranasally, intragastrically, topically, sublingually, vaginally, or rectally, but is not limited thereto.

Additionally, the peptide may be used by mixing with various pharmaceutically acceptable carriers such as physiological saline or organic solvents. For increasing stability or absorptivity, carbohydrates such as glucose, sucrose, or dextrans, antioxidants such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins, or other stabilizers may be used as pharmaceutical drugs.

The administration dose and frequency of the pharmaceutical composition of the present invention are determined by the type of active ingredient(s), together with various factors such as the disease to be treated, administration route, patient's age, gender, and body weight, and severity of the disease.

The total effective dose of the composition of the present invention may be administered to a patient in a single dose or may be administered for a long period of time in multiple doses according to a fractionated treatment protocol. In the pharmaceutical

composition of the present invention, the content of active ingredient(s) may vary depending on the disease severity. Specifically, the total daily dose of the peptide of the present invention may be about 0.0001 mg to 500 mg per 1 kg of body weight of a patient. However, the effective dose of the peptide is determined considering various factors including patient's age, body weight, health conditions, gender, disease severity, diet, and excretion rate, in addition to administration route and treatment frequency of the pharmaceutical composition. In this regard, those skilled in the art may easily determine the effective dose suitable for the particular use of the pharmaceutical composition of the present invention. The pharmaceutical composition according to the present invention is not particularly limited to the formulation and administration route and mode, as long as it shows the effects of the present invention.

The pharmaceutical composition of the present invention shows excellent *in vivo* duration of efficacy and titer, and thus the number and frequency of administration of the pharmaceutical preparation of the present invention can be significantly reduced.

Still another aspect of the present invention provides a method for treating a target disease, which includes administering the isolated peptide or a composition containing the isolated peptide to a subject in need thereof. The target disease may be a metabolic syndrome.

The isolated peptide, or the composition containing the same, the metabolic syndrome and treatment thereof are the same as explained above.

As used herein, the term "subject" refers to a subject suspected of having a metabolic syndrome, and the subject suspected of having a metabolic syndrome refers to mammals including humans, rats, cattle, *etc.*, which have or are at the risk of developing the metabolic syndrome, but any subject which can be treated with the above peptide of the present invention or the composition containing the same is included without limitation.

The therapeutic method of the present invention may include administering a pharmaceutically effective amount of the pharmaceutical composition containing the peptide. The total daily dose of the composition may be determined within the scope of appropriate medical judgment by a physician, and the composition may be administered once or several times in divided doses a day. However, for the purpose of the present invention, the specific

therapeutically effective dose of the composition for any particular patient is preferably applied differently depending on various factors including the kind and degree of response to be achieved, specific compositions including whether other agents are occasionally used therewith, the patient's age, body weight, health condition, gender and diet, the time and route of administration, the secretion rate of the composition, the duration of treatment, other drugs used in combination or simultaneously with the specific compositions, and similar factors well-known in the medical field.

Still another aspect of the present invention provides the use of the isolated peptide or composition containing the peptide in the preparation of a medicament. The medicament may be for preventing or treating metabolic syndrome.

The isolated peptide or composition and metabolic syndrome are the same as explained above.

Hereinafter, the present invention will be described in more detail with reference to the following Examples. However, these Examples are for illustrative purposes only and the invention is not intended to be limited by these Examples.

Example 1: Preparation of triple agonists

Triple agonists showing activities to all of GLP-1, GIP, and glucagon receptors were prepared and their amino acid sequences are shown in Table 1 below.

[Table 1]

SEQ ID NO	Sequence	Information
1	HXQGTFTSDVSSYLDGQA AKEFIAWL VKGC	
2	HXQGTFTSDVSSYLDGQA QKEFIAWL VKGC	
3	HXQGTFTSDVSSYLLGQA AKQFIAWL VKGGG PSSGAPPPSC	
4	HXQGTFTSDVSSYLLGQQQKEFIAWL VKGC	
5	HXQGTFTSDVSSYLLGQQQKEFIAWL VKGGG PSSGAPPPSC	

6	HXQGTFTSDVSSYLDGQAAKEFVAWLLKGC	
7	HXQGTFTSDVSKYLDGQAAKEFVAWLLKGC	
8	HXQGTFTSDVSKYLDGQAAQEFVAWLLKGC	
9	HXQGTFTSDVSKYLDGQAAQEFVAWLLAGC	
10	HXQGTFTSDVSKYLDGQAAQEFVAWLLAGG GPSSGAPPPSC	
11	CAGEGTFTSDLSKYLD S RRQQLFVQWLKAGG PSSGAPPPSHG	
12	CAGEGTFISDLSKYMDEQAVQLFVEWLMAGG PSSGAPPPSHG	
13	CAGEGTFISDYSIQLDEIAVQDFVEWLLAQKP SSGAPPPSHG	
14	CAGQGTFTSDYSIQLDEIAVRDFVEWLKNGGP SSGAPPPSHG	
15	CAGQGTFTSDLSKQMDEEAVRLFIEWLKNGG PSSGAPPPSHG	
16	CAGQGTFTSDLSKQMDSEAQQLFIEWLKNGG PSSGAPPPSHG	
17	CAGQGTFTSDLSKQMDEERAREFIEWLLAQKP SSGAPPPSHG	
18	CAGQGTFTSDLSKQMDSERAREFIEWLKNTGP SSGAPPPSHG	
19	CAGQGTFTSDLSIQYDSEHQ R DFIEWLKDTGP SSGAPPPSHG	
20	CAGQGTFTSDLSIQYEEEAQQDFVEWLKDTGP SSGAPPPSHG	
21	YXQGTFTSDYSKYLD E CRA K EFVQWLLDHHP SSGQPPPS	Ring formation
22	YXQGTFTSDYSKCLD E KRA K EFVQWLLDHHP SSGQPPPS	Ring formation
23	YXQGTFTSDYSKYLD E CRA K EFVQWLLAQKG	Ring

	KKNDWKHNIT	formation
24	YXQGTFTSDYSKYLDE <u>C</u> RA <u>K</u> EFVQWLKNGGP SSGAPPPS	Ring formation
25	HXQGTFTSDCSKYLDERAAQDFVQWLLDGGP SSGAPPPS	
26	HXQGTFTSDCSKYLDSRAAQDFVQWLLDGGP SSGAPPPS	
27	HXQGTFTSDYSKYLDERACQDFVQWLLDQGG PSSGAPPPS	
28	HXQGTFTSDYSKYLDEKRAQEFVCWLLAQKG KKNDWKHNIT	
29	HXQGTFTSDYSKYLDE <u>K</u> AA <u>K</u> EFVQWLLNTC	Ring formation
30	HXQGTFTSDYSKYLDE <u>K</u> AQ <u>K</u> EFVQWLLDTC	Ring formation
31	HXQGTFTSDYSKYLDE <u>K</u> AC <u>K</u> EFVQWLLAQ	Ring formation
32	HXQGTFTSDYSKYLDE <u>K</u> AC <u>K</u> DFVQWLLDGGP SSGAPPPS	Ring formation
33	HXQGTFTSDYSIAMDE <u>I</u> HQ <u>K</u> DFVNWLLAQKC	Ring formation
34	HXQGTFTSDYSKYLDE <u>K</u> RQ <u>K</u> EFVNWLLAQKC	Ring formation
35	HXQGTFTSDYSIAMDE <u>I</u> HQ <u>K</u> DFVNWLLNTKC	Ring formation
36	HXQGTFTSDYSKYLCE <u>K</u> RQ <u>K</u> EFVQWLLNGGP SSGAPPPSG	Ring formation
37	HXQGTFTSDYSKYLDE <u>C</u> RQ <u>K</u> EFVQWLLNGGP SSGAPPPSG	Ring formation
38	CAXQGTFTSDKSSYLDERAAQDFVQWLLDGG PSSGAPPPSS	

39	HXQGTFTSDYSKYLDGQHAQCFVAWLLAGG GPSSGAPPPS	
40	HXQGTFTSDKSKYLDERACQDFVQWLLDGGP SSGAPPPS	
41	HXQGTFTSDKSKYLDECAAQDFVQWLLDGGP SSGAPPPS	
42	YXQGTFTSDYSKYLDE <u>E</u> KRA <u>K</u> EFVQWLLDHHP SSGQPPPS	Ring formation
43	YXQGTFTSDYSKYLDE <u>E</u> KRA <u>K</u> EFVQWLLDHHC SSGQPPPS	Ring formation
44	HGQGTFTSDCSKQLDGQAAQEFVAWLLAGGP SSGAPPPS	
45	HGQGTFTSDCSKYMDGQAAQDFVAWLLAGG PSSGAPPPS	
46	HGQGTFTSDCSKYLDEQHAQEFVAWLLAGGP SSGAPPPS	
47	HGQGTFTSDCSKYLDGQRAQEFVAWLLAGGP SSGAPPPS	
48	HGQGTFTSDCSKYLDGQRAQDFVNWLLAGGP SSGAPPPS	
49	CAXQGTFTSDYSICMDE <u>I</u> HQ <u>K</u> DFVNWLLNTK	Ring formation
50	HXQGTFTSDYSKYLDE <u>E</u> KRA <u>K</u> EFVQWLLDHHP SSGQPPPS	Ring formation
51	HXQGTFTSDYSKYLDE <u>E</u> KRQ <u>K</u> EFVQWLLNTC	Ring formation
52	HXQGTFTSDYSKYLDE <u>E</u> KRQ <u>K</u> EFVQWLLDTC	Ring formation
53	HXEGTFTSDYSIAMDE <u>I</u> HQ <u>K</u> DFVNWLLAQC	Ring formation
54	HXEGTFTSDYSIAMDE <u>I</u> HQ <u>K</u> DFVDWLLAEC	Ring

		formation
55	HXQGTFTSDYSIAMDE <u>I</u> HQ <u>K</u> DFVNWLLAQC	Ring formation
56	HXQGTFTSDYSKYLDE <u>K</u> RQ <u>K</u> EFVNWLLAQC	Ring formation
57	HXQGTFTSDYSIAMDE <u>I</u> HQ <u>K</u> DFVNWLLNTC	Ring formation
58	HXQGTFTSDYSKYLDE <u>K</u> RQ <u>K</u> EFVQWLLNTKC	Ring formation
59	CAXQGTFTSDYSICMDE <u>K</u> HQ <u>K</u> DFVNWLLNTK	Ring formation
60	CAXQGTFTSDYSIAMDE <u>K</u> H <u>C</u> KDFVNWLLNTK	Ring formation
61	CAXQGTFTSDYSIAMDE <u>I</u> A <u>C</u> KDFVNWLLNTK	Ring formation
62	CAXQGTFTSDKSKYLDERAAQDFVQWLLDGG PSSGAPPPS	
63	CAXQGTFTSDCSKYLDERAAQDFVQWLLDGG PSSGAPPPS	
64	YXQGTFTSDYSKYLDE <u>C</u> AA <u>K</u> EFVQWLLDHHP SSGQPPPS	Ring formation
65	HXQGTFTSDYSKCLDE <u>K</u> RA <u>K</u> EFVQWLLDHHP SSGQPPPS	Ring formation
66	YXQGTFTSDYSKYLDE <u>C</u> RA <u>K</u> DFVQWLLDHHP SSGQPPPS	Ring formation
67	YXQGTFTSDYSKYLDE <u>C</u> AA <u>K</u> DFVQWLLDHHP SSGQPPPS	Ring formation
68	YXQGTFTSDYSKCLDE <u>K</u> AA <u>K</u> EFVQWLLDHHP SSGQPPPS	Ring formation
69	YXQGTFTSDYSKCLDE <u>R</u> AA <u>K</u> EFVQWLLDHHP SSGQPPPS	Ring formation

70	YXQGTFTSDYSKCLDE <u>E</u> KRA <u>K</u> DFVQWLLDHHP SSGQPPPS	Ring formation
71	YXQGTFTSDYSKYLD <u>E</u> RAC <u>K</u> DFVQWLLDHHP SSGQPPPS	Ring formation
72	YXQGTFTSDCSKYLD <u>E</u> RAA <u>K</u> DFVQWLLDHHP SSGQPPPS	Ring formation
73	CAXQGTFTSDYSKYLD <u>E</u> CRA <u>K</u> EFVQWLLDHH PSSGQPPPS	Ring formation
74	CAXQGTFTSDYSKCLDE <u>E</u> KRA <u>K</u> EFVQWLLDHH PSSGQPPPS	Ring formation
75	YXQGTFTSDYSKYLD <u>E</u> KAA <u>K</u> EFVQWLLDHHP SSGQPPPS	Ring formation
76	YXQGTFTSDYSKYLD <u>E</u> KRA <u>K</u> DFVQWLLDHHP SSGQPPPS	Ring formation
77	YXQGTFTSDYSKYLD <u>E</u> KAA <u>K</u> DFVQWLLDHHP SSGQPPPS	Ring formation
78	HXQGTFTSDYSKYLD <u>E</u> KRQ <u>K</u> EFVQWLLDTKC	Ring formation
79	HXEGTFTSDYSIAMDE <u>E</u> IHQ <u>K</u> DFVNWLLAQKC	Ring formation
80	HXEGTFTSDYSIAMDE <u>E</u> IHQ <u>K</u> DFVDWLLAEKC	Ring formation
81	CAXQGTFTSDYSKYLD <u>E</u> KRQ <u>K</u> EFVQWLLNTC	Ring formation
82	CAXQGTFTSDYSKYLD <u>E</u> KRQ <u>K</u> EFVQWLLDTC	Ring formation
83	CAXEGTFTSDYSIAMDE <u>E</u> IHQ <u>K</u> DFVNWLLAQC	Ring formation
84	CAXEGTFTSDYSIAMDE <u>E</u> IHQ <u>K</u> DFVDWLLAEC	Ring formation
85	CAXQGTFTSDYSIAMDE <u>E</u> IHQ <u>K</u> DFVNWLLAQC	Ring

		formation
86	CAXQGTFTSDYSKYLD <u>E</u> KRQ <u>K</u> EFVNWLLAQC	Ring formation
87	CAXQGTFTSDYSIAMD <u>E</u> IHQ <u>K</u> DFVNWLLNTC	Ring formation
88	CAXQGTFTSDYSKYLD <u>E</u> KRQ <u>K</u> EFVQWLLNTKC	Ring formation
89	CAXQGTFTSDYSKYLD <u>E</u> KRQ <u>K</u> EFVQWLLDTKC	Ring formation
90	CAXEGTFTSDYSIAMD <u>E</u> IHQ <u>K</u> DFVNWLLAQKC	Ring formation
91	CAXEGTFTSDYSIAMD <u>E</u> IHQ <u>K</u> DFVDWLLAEKC	Ring formation
92	CAXQGTFTSDYSIAMD <u>E</u> IHQ <u>K</u> DFVNWLLAQKC	Ring formation
93	CAXQGTFTSDYSKYLD <u>E</u> KRQ <u>K</u> EFVNWLLAQKC	Ring formation
94	CAXQGTFTSDYSIAMD <u>E</u> IHQ <u>K</u> DFVNWLLNTKC	Ring formation
95	YXQGTFTSDYSKYLD <u>E</u> KRA <u>K</u> EFVQWLLCHHP SSGQPPPS	Ring formation
96	YXQGTFTSDYSKYLD <u>E</u> KRA <u>K</u> EFVQWLLDHCP SSGQPPPS	Ring formation
97	YXQGTFTSDYSKYLD <u>E</u> KRA <u>K</u> EFVQWLLDCHP SSGQPPPS	Ring formation
98	YXQGTFTSDYSKALD <u>E</u> KAA <u>K</u> EFVNWLLDHHP SSGQPPPS	Ring formation
99	YXQGTFTSDYSKALD <u>E</u> KAA <u>K</u> DFVNWLLDHHP SSGQPPPS	Ring formation
100	YXQGTFTSDYSKALD <u>E</u> KAA <u>K</u> EFVQWLLDQHP SSGQPPPS	Ring formation

101	Y X Q G T F T S D Y S K A L D <u>E</u> K A A <u>K</u> E F V N W L L D Q H P S S G Q P P P S C	Ring formation
102	Y X Q G T F T S D Y S K A L D <u>E</u> K A A <u>K</u> D F V N W L L D Q H P S S G Q P P P S C	Ring formation

In the sequences described in Table 1, the amino acid represented by X represents aminoisobutyric acid (Aib), which is a non-natural amino acid, and the underlined amino acids represent the formation of a ring between the underlined amino acids. Additionally, in Table 1, CA represents 4-imidazoacetyl and Y represents tyrosine.

Example 2: Measurement of *in vitro* activities of triple agonists

The activities of the triple agonists prepared in Example 1 were measured by a method of measuring *in vitro* cellular activities using cell lines, where a GLP-1 receptor, a glucagon (GCG) receptor, and a GIP receptor are transformed, respectively.

Each of the cell lines above is one in which the genes for human GLP-1 receptor, human GCG receptor, and human GIP receptor were transformed in Chinese hamster ovary (CHO), respectively, and can be expressed therein, and is thus suitable for the measurement of the activities of GLP-1, GCG, and GIP. Accordingly, the activity for each part was measured using the respective transformed cell line.

For the measurement of the GLP-1 activity of the triple agonists prepared in Example 1, human GLP-1 was subjected to a 4-fold serial dilution from 50 nM to 0.000048 nM and the triple agonists prepared in Example 1 were subjected to a 4-fold serial dilution from 400 nM to 0.00038 nM.

The culture solution was removed from the cultured CHO cells, in which the human GLP-1 receptor was expressed, and each of the serially-diluted materials was added to the CHO cells in an amount of 5 μ L, respectively. Then, a buffer solution containing cAMP antibody was added thereto in an amount of 5 μ L and cultured at room temperature for 15 minutes. Then, a detection mix containing a cell lysis buffer was added thereto in an amount of 10 μ L for the lysis of the cells and reacted at room temperature for 90 minutes. The cell lysates, upon completion of the reaction, were applied to LANCE cAMP kit

(PerkinElmer, USA) to calculate the EC₅₀ value via accumulated cAMP, and the values were compared with each other. The relative potencies compared to human GLP-1 are shown in Table 2 below.

For the measurement of the GCG activity of the triple agonists prepared in Example 1, human GCG was subjected to a 4-fold serial dilution from 50 nM to 0.000048 nM and the triple agonists prepared in Example 1 were subjected to a 4-fold serial dilution from 400 nM to 0.00038 nM.

The culture solution was removed from the cultured CHO cells, in which the human GCG receptor was expressed, and each of the serially-diluted materials was added to the CHO cells in an amount of 5 μ L, respectively. Then, a buffer solution containing cAMP antibody was added thereto in an amount of 5 μ L and cultured at room temperature for 15 minutes. Then, a detection mix containing a cell lysis buffer was added thereto in an amount of 10 μ L for the lysis of the cells and reacted at room temperature for 90 minutes. The cell lysates, upon completion of the reaction, were applied to LANCE cAMP kit (PerkinElmer, USA) to calculate the EC₅₀ value via accumulated cAMP, and the values were compared with each other. The relative potencies compared to human GCG are shown in Table 2 below.

For the measurement of the GIP activity of the triple agonists prepared in Example 1, human GIP was subjected to a 4-fold serial dilution from 50 nM to 0.000048 nM and the triple agonists prepared in Example 1 were subjected to a 4-fold serial dilution from 400 nM to 0.00038 nM.

The culture solution was removed from the cultured CHO cells, in which the human GIP receptor was expressed, and each of the serially-diluted materials was added to the CHO cells in an amount of 5 μ L, respectively. Then, a buffer solution containing cAMP antibody was added thereto in an amount of 5 μ L and cultured at room temperature for 15 minutes. Then, a detection mix containing a cell lysis buffer was added thereto in an amount of 10 μ L for the lysis of the cells and reacted at room temperature for 90 minutes. The cell lysates, upon completion of the reaction, were applied to LANCE cAMP kit (PerkinElmer, USA) to calculate the EC₅₀ value via accumulated cAMP, and the values were compared with each other. The relative potencies compared to human GIP are shown in Table 2 below.

[Table 2]

	<i>In vitro</i> activity compared to native peptide (%)		
SEQ ID NO	<i>vs.</i> GLP-1	<i>vs.</i> Glucagon	<i>vs.</i> GIP
1	3.2	< 0.1	< 0.1
2	5.9	< 0.1	< 0.1
3	1.8	< 0.1	< 0.1
4	8.5	< 0.1	< 0.1
5	42.1	< 0.1	< 0.1
6	17.0	< 0.1	< 0.1
7	13.7	< 0.1	< 0.1
8	14.2	0.10	< 0.1
9	32.1	0.13	< 0.1
10	46.0	< 0.1	< 0.1
11	1.4	< 0.1	< 0.1
12	0.4	< 0.1	< 0.1
13	< 0.1	< 0.1	< 0.1
14	28.0	< 0.1	< 0.1
15	79.2	< 0.1	< 0.1
16	2.1	< 0.1	< 0.1
17	0.2	< 0.1	< 0.1
18	< 0.1	< 0.1	< 0.1

19	< 0.1	< 0.1	< 0.1
20	< 0.1	< 0.1	< 0.1
21	17.8	267	22.7
22	20.1	140	59.7
23	4.01	9.3	< 0.1
24	41.2	9.3	< 0.1
25	82.6	0.1	< 0.1
26	64.5	0.2	< 0.1
27	83.1	0.8	0.9
28	17.2	1.6	< 0.1
29	38.5	6.0	< 0.1
30	142	0.7	0.8
31	135	2.2	2.4
32	151	1.7	8.8
33	24.5	< 0.1	10.4
34	19.1	0.92	0.6
35	7.5	< 0.1	1.3
36	37.4	0.39	0.2
37	236	6.21	2.2
38	2.3	-	-
39	13.9	0.53	< 0.1
40	75.2	< 0.1	< 0.1

41	34.3	< 0.1	< 0.1
42	33.9	205.8	7.8
43	12.6	88.4	3.70
44	1.3	< 0.1	< 0.1
45	6.6	< 0.1	< 0.1
46	1.4	< 0.1	< 0.1
47	2.4	< 0.1	< 0.1
48	1.5	< 0.1	< 0.1
49	29.8	< 0.1	3.3
50	67.4	50.5	2.7
51	14.4	2.0	0.1
52	44.1	7.5	0.3
53	161	8.4	1.3
54	30.6	1.4	0.1
55	27.1	0.7	2.4
56	57.9	4.9	0.8
57	11.7	< 0.1	0.3
58	39.1	2.6	0.2
59	40.3	< 0.1	4.0
60	106.2	< 0.1	8.2
61	59.8	< 0.1	2.8
62	5.2	< 0.1	< 0.1

63	15.3	< 0.1	< 0.1
64	64.6	60.1	92.9
65	95.4	25.2	11.6
66	15.8	172	17.2
67	28.5	46.2	39.8
68	27.9	8.8	107
69	24.3	9.6	62.8
70	15.1	71.3	64.4
71	90.1	12.7	94.7
72	11.5	1.0	1.6
73	22.6	5.4	3.0
74	12.9	0.9	1.0
75	35.1	8.5	18.0
76	10.3	47.6	11.7
77	38.7	12.2	35.5
78	51.0	14.0	0.12
79	41.5	4.9	1.4
80	8.1	0.0	0.1
81	7.8	0.3	< 0.1
82	9.5	1.1	< 0.1
83	47.3	1.3	0.4
84	4.2	< 0.1	< 0.1

85	4.3	< 0.1	0.3
86	28.4	0.4	0.2
87	0.9	< 0.1	< 0.1
88	9.6	0.3	< 0.1
89	7.1	0.7	< 0.1
90	7.4	< 0.1	< 0.1
91	31.9	16.8	0.3
92	0.8	< 0.1	0.4
93	5.7	0.3	0.7
94	0.5	< 0.1	< 0.1
95	2.1	0.4	< 0.1
96	34.4	194.8	5.2
97	10.5	62.8	2.6
98	28.1	8.2	47.1
99	20.9	14.9	57.7
100	42.2	12.7	118.5
101	23.2	13.9	40.1
102	23.3	29.5	58.0

The novel glucagon analogs prepared above have the function of triple agonists which can activate all of GLP-1 receptors, GIP receptors, and glucagon receptors, and thus the glucagon analogs can be used as a therapeutic material for treating patients with metabolic syndrome including diabetes and obesity.

From the foregoing, a skilled person in the art to which the present invention pertains will be able to understand that the present invention may be embodied in other specific forms without modifying the technical concepts or essential characteristics of the present invention. In this regard, the exemplary embodiments disclosed herein are only for illustrative purposes and should not be construed as limiting the scope of the present invention. On the contrary, the present invention is intended to cover not only the exemplary embodiments but also various alternatives, modifications, equivalents, and other embodiments that may be included within the spirit and scope of the present invention as defined by the appended claims.

Claims

1. An isolated peptide having the function of a triple agonist which activates a glucagon receptor, a glucagon-like peptide-1 (GLP-1) receptor, and a glucose-dependent insulintropic polypeptide (GIP) receptor, wherein the isolated peptide comprises an amino acid sequence represented by General Formula 1 below:

Xaa1-Xaa2-Xaa3-Gly-Thr-Phe-Xaa7-Ser-Asp-Xaa10-Ser-Xaa12-Xaa13-Xaa14-Xaa15-Xaa16-Xaa17-Xaa18-Xaa19-Xaa20-Xaa21-Phe-Xaa23-Xaa24-Trp-Leu-Xaa27-Xaa28-Xaa29-Xaa30-R1 (General Formula 1, SEQ ID NO: 103)

wherein, in General Formula 1,

Xaa1 is histidine (His, H), 4-imidazoacetyl (CA), or tyrosine (Tyr, Y);

Xaa2 is glycine (Gly, G), or aminoisobutyric acid (Aib);

Xaa3 is glutamic acid (Glu, E) or glutamine (Gln, Q);

Xaa7 is threonine (Thr, T) or isoleucine (Ile, I);

Xaa10 is leucine (Leu, L), tyrosine (Tyr, Y), lysine (Lys, K), cysteine (Cys, C), or valine (Val, V);

Xaa12 is lysine (Lys, K), serine (Ser, S), or isoleucine (Ile, I);

Xaa13 is glutamine (Gln, Q), tyrosine (Tyr, Y), alanine (Ala, A), or cysteine (Cys, C);

Xaa14 is leucine (Leu, L), methionine (Met, M), or tyrosine (Tyr, Y);

Xaa15 is cysteine (Cys, C), aspartic acid (Asp, D), glutamic acid (Glu, E), or leucine (Leu, L);

Xaa16 is glycine (Gly, G), glutamic acid (Glu, E), or serine (Ser, S);

Xaa17 is glutamine (Gln, Q), arginine (Arg, R), isoleucine (Ile, I), glutamic acid (Glu, E), cysteine (Cys, C), or lysine (Lys, K);

Xaa18 is alanine (Ala, A), glutamine (Gln, Q), arginine (Arg, R), or histidine (His, H);

Xaa19 is alanine (Ala, A), glutamine (Gln, Q), cysteine (Cys, C), or valine (Val, V);

Xaa20 is lysine (Lys, K), glutamine (Gln, Q), or arginine (Arg, R);

Xaa21 is glutamic acid (Glu, E), glutamine (Gln, Q), leucine (Leu, L), cysteine (Cys, C), or aspartic acid (Asp, D);

Xaa23 is valine (Val, V);

Xaa24 is alanine (Ala, A), glutamine (Gln, Q), cysteine (Cys, C), asparagine (Asn, N), aspartic acid (Asp, D), or glutamic acid (Glu, E);

Xaa27 is valine (Val, V), leucine (Leu, L), or lysine (Lys, K);

Xaa28 is cysteine (Cys, C), lysine (Lys, K), alanine (Ala, A), asparagine (Asn, N), or aspartic acid (Asp, D);

Xaa29 is cysteine (Cys, C), glycine (Gly, G), glutamine (Gln, Q), threonine (Thr, T), glutamic acid (Glu, E), or histidine (His, H);

Xaa30 is cysteine (Cys, C), glycine (Gly, G), lysine (Lys, K), or histidine (His, H), or is absent; and

R1 is cysteine (Cys, C), GKKNWVKHNIT (SEQ ID NO: 106), m-SSGAPPPS-n (SEQ ID NO: 107), or m-SSGQPPPS-n (SEQ ID NO: 108), or is absent;

wherein:

m is -Cys-, -Pro-, or -Gly-Pro-; and

n is -Cys-, -Gly-, -Ser-, or -His-Gly-, or is absent.

2. The isolated peptide of claim 1, wherein Xaa14 is leucine or methionine; and Xaa15 is cysteine, aspartic acid, or leucine.

3. The isolated peptide of claim 1,

wherein, in General Formula 1,

Xaa2 is glycine, or Aib;

Xaa7 is threonine,

Xaa10 is tyrosine, cysteine, or valine;

Xaa12 is lysine or isoleucine;

Xaa13 is tyrosine, alanine, glutamine, or cysteine;

Xaa14 is leucine, tyrosine, or methionine;

Xaa15 is cysteine, leucine, glutamic acid, or aspartic acid;

Xaa17 is glutamine, arginine, isoleucine, cysteine, glutamic acid, or lysine;

Xaa18 is alanine, glutamine, arginine, or histidine;

Xaa19 is alanine, glutamine, valine, or cysteine;

Xaa20 is lysine, arginine, or glutamine;

Xaa21 is glutamic acid, glutamine, leucine, cysteine, or aspartic acid;

Xaa23 is valine;

Xaa24 is cysteine, alanine, glutamine, asparagine, glutamic acid, or aspartic acid; and

Xaa27 is leucine or lysine.

4. The isolated peptide of claim 1, wherein the peptide comprises an amino acid sequence represented by General Formula 2 below:

Xaa1-Xaa2-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Xaa10-Ser-Lys-Xaa13-Xaa14-Xaa15-Xaa16-Xaa17-Xaa18-Xaa19-Xaa20-Xaa21-Phe-Xaa23-Xaa24-Trp-Leu-Leu-Xaa28-Xaa29-Xaa30-Xaa31-Ser-Ser-Gly-Gln-Pro-Pro-Pro-Ser-Xaa40 (General Formula 2, SEQ ID NO: 104)

wherein, in General Formula 2,

Xaa1 is 4-imidazoacetyl, histidine, or tyrosine;

Xaa2 is glycine, or Aib;

Xaa10 is tyrosine or cysteine;

Xaa13 is alanine, glutamine, tyrosine, or cysteine;

Xaa14 is leucine, methionine, or tyrosine;

Xaa15 is aspartic acid, glutamic acid, or leucine;

Xaa16 is glycine, glutamic acid, or serine;

Xaa17 is glutamine, arginine, isoleucine, glutamic acid, cysteine, or lysine;

Xaa18 is alanine, glutamine, arginine, or histidine;

Xaa19 is alanine, glutamine, cysteine, or valine;

Xaa20 is lysine, glutamine, or arginine;

Xaa21 is cysteine, glutamic acid, glutamine, leucine, or aspartic acid;
Xaa23 is valine;
Xaa24 is cysteine, alanine, glutamine, asparagine, or glutamic acid;
Xaa28 is lysine, cysteine, asparagine, or aspartic acid;
Xaa29 is glycine, glutamine, cysteine, or histidine;
Xaa30 is cysteine, glycine, lysine, or histidine;
Xaa31 is proline or cysteine; and
Xaa40 is cysteine or is absent.

5. The isolated peptide of claim 1,
wherein, in General Formula 1,
Xaa2 is glycine, or Aib;
Xaa7 is threonine;
Xaa10 is tyrosine, cysteine, or valine;
Xaa12 is lysine or isoleucine;
Xaa13 is tyrosine, alanine, or cysteine;
Xaa14 is leucine or methionine;
Xaa15 is cysteine or aspartic acid;
Xaa17 is glutamine, arginine, isoleucine, cysteine, or lysine;
Xaa18 is alanine, arginine, or histidine;
Xaa19 is alanine, glutamine, or cysteine;
Xaa20 is lysine or glutamine;
Xaa21 is glutamic acid, cysteine, or aspartic acid;
Xaa23 is valine;
Xaa24 is alanine, glutamine, cysteine, asparagine, or aspartic acid; and
Xaa27 is leucine or lysine.

6. The isolated peptide of claim 4,
wherein, in General Formula 2,
Xaa13 is alanine, tyrosine, or cysteine;
Xaa15 is aspartic acid or glutamic acid;
Xaa17 is glutamine, arginine, cysteine, or lysine;
Xaa18 is alanine, arginine, or histidine;
Xaa21 is cysteine, glutamic acid, glutamine, or aspartic acid;
Xaa23 is valine;
Xaa24 is cysteine, glutamine, or asparagine;
Xaa28 is cysteine, asparagine, or aspartic acid;
Xaa29 is glutamine, cysteine, or histidine; and
Xaa30 is cysteine, lysine, or histidine.

7. The isolated peptide of claim 1,
wherein, in General Formula 1,
Xaa2 is Aib;
Xaa7 is threonine;
Xaa10 is tyrosine or cysteine;

Xaa12 is lysine or isoleucine;
Xaa13 is tyrosine, alanine, or cysteine;
Xaa14 is leucine or methionine;
Xaa15 is cysteine or aspartic acid;
Xaa16 is glutamic acid;
Xaa17 is arginine, isoleucine, cysteine, or lysine;
Xaa18 is alanine, arginine, or histidine;
Xaa19 is alanine, glutamine, or cysteine;
Xaa20 is lysine or glutamine;
Xaa21 is glutamic acid or aspartic acid;
Xaa23 is valine;
Xaa24 is glutamine, asparagine, or aspartic acid;
Xaa27 is leucine; and
Xaa28 is cysteine, alanine, asparagine, or aspartic acid.

8. The isolated peptide of claim 1,
wherein, in General Formula 1,
Xaa1 is histidine or 4-imidazoacetyl;
Xaa2 is Aib;
Xaa3 is glutamine;
Xaa7 is threonine;
Xaa10 is tyrosine;
Xaa12 is isoleucine;
Xaa13 is alanine or cysteine;
Xaa14 is methionine;
Xaa15 is aspartic acid;
Xaa16 is glutamic acid;
Xaa17 is isoleucine or lysine;
Xaa18 is alanine or histidine;
Xaa19 is glutamine or cysteine;
Xaa20 is lysine;
Xaa21 is aspartic acid;
Xaa23 is valine;
Xaa24 is asparagine;
Xaa27 is leucine;
Xaa28 is alanine or asparagine;
Xaa29 is glutamine or threonine; and
Xaa30 is cysteine or lysine, or is absent.

9. The isolated peptide of claim 1, wherein the peptide comprises an amino acid sequence represented by General Formula 3 below:

Xaa1-Xaa2-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Xaa13-Leu-Asp-Glu-Xaa17-Xaa18-Xaa19-Lys-Xaa21-Phe-Val-Xaa24-Trp-Leu-Leu-Xaa28-Xaa29-Xaa30-Xaa31-Ser-Ser-Gly-Gln-Pro-Pro-Pro-Ser-Xaa40 (General Formula 3, SEQ ID NO: 105),

wherein, in General Formula 3,
Xaa1 is histidine or tyrosine;
Xaa2 is Aib;
Xaa13 is alanine, tyrosine, or cysteine;
Xaa17 is arginine, cysteine, or lysine;
Xaa18 is alanine or arginine;
Xaa19 is alanine or cysteine;
Xaa21 is glutamic acid or aspartic acid;
Xaa24 is glutamine or asparagine;
Xaa28 is cysteine or aspartic acid;
Xaa29 is cysteine, histidine, or glutamine;
Xaa30 is cysteine or histidine;
Xaa31 is proline or cysteine; and
Xaa40 is cysteine or is absent.

10. The isolated peptide of claim 1, wherein R1 is cysteine, GKKNDWKHNIT (SEQ ID NO: 106), CSSGQPPPS (SEQ ID NO: 109), GPSSGAPPPS (SEQ ID NO: 110), GPSSGAPPPSC (SEQ ID NO: 111), PSSGAPPPS (SEQ ID NO: 112), PSSGAPPPSG (SEQ ID NO: 113), PSSGAPPPSHG (SEQ ID NO: 114), PSSGAPPPSS (SEQ ID NO: 115), PSSGQPPPS (SEQ ID NO: 116), or PSSGQPPPS (SEQ ID NO: 117), or is absent.

11. The isolated peptide according to any one of claims 1 to 10, wherein, in General Formulas 1 to 3, the 16th amino acid and the 20th amino acid from the N-terminus together form a ring.

12. The isolated peptide of claim 1, wherein the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 6 to 11, 13, 14, and 20 to 102.

13. An isolated peptide having the function of a triple agonist which activates a glucagon receptor, a glucagon-like peptide-1 (GLP-1) receptor, and a glucose-dependent insulintropic polypeptide (GIP) receptor, wherein the isolated peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 1 to 102.

14. The isolated peptide according to claim 13, wherein the isolated peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 21, 22, 27, 30-32, 34, 36-38, 42, 43, 50-56, 58, 64-80, 83, 86, 91, 93, and 96-102.

15. The isolated peptide according to any one of claims 1 to 10, 13 and 14, wherein the peptide has an increased in vivo half-life compared to that of any one of native GLP-1, native glucagon, and native GIP.

16. The isolated peptide according to any one of claims 1 to 10, 13 and 14, wherein the C-terminus of the peptide is amidated.
17. A polynucleotide encoding the peptide according to any one of claims 1 to 10, 13 and 14.
18. Use of a peptide according to any one of claims 1 to 10, 13 and 14 in the manufacture of a medicament for preventing or treating a metabolic syndrome.
19. The use of claim 18, wherein the metabolic syndrome comprises impaired glucose tolerance, hypercholesterolemia, dyslipidemia, obesity, diabetes, hypertension, arteriosclerosis due to dyslipidemia, atherosclerosis, arteriosclerosis, or coronary heart disease.