

the non-terminal, internal residue of a physiologically active protein or peptide, via a non-peptidyl linker.

[12] Another object of the present invention is to provide a method for preparing the conjugate of the physiologically active protein or peptide, in which the carrier is bound to the non-terminal, internal residue of the physiologically active protein or peptide.

[13]

【Solution to Problem】

[14] In one aspect, the present invention provides a method for decreasing immunogenicity of a physiologically active protein or peptide as compared to that of a physiologically active protein or peptide to which a carrier is not bound, which comprises inhibiting the mechanism in which the desired protein or peptide acts as an antigen by binding a carrier to a non-terminal, internal residue of the physiologically active protein or peptide, wherein the physiologically active protein or peptide is selected from the group consisting of an exendin-4, an exendin-4 derivative, a GLP-1, a GLP-1 agonist, an insulin and a GLP-1/glucagon dual agonist; and wherein the carrier is a polyethylene glycol, or an immunoglobulin Fc region. In another aspect, the present invention provides a composition, comprising a conjugate of a physiologically active protein or peptide in which a carrier is bound to the non-terminal, internal residue of a physiologically active protein or peptide, via a non-peptidyl linker, wherein the conjugate inhibits the mechanism in which the desired protein or peptide acts as an antigen and exhibits decreased immunogenicity as compared to that of the physiologically active protein or peptide to which the carrier is not bound, wherein the physiologically active protein or peptide is selected from the group consisting of an exendin-4, an exendin-4 derivative, a GLP-1, a GLP-1 agonist, an insulin and a GLP-1/glucagon dual agonist; and wherein the carrier is a polyethylene glycol, or an immunoglobulin Fc region. Also disclosed herein is a method for decreasing immunogenicity of a physiologically active protein or peptide as compared to that of a physiologically active protein or peptide to which a carrier is not bound, which comprises binding a carrier to the non-terminal, internal residue of the physiologically active protein or peptide.

[15] In one specific example, the above carrier is characterized in that it is selected from the group consisting of a polyethylene glycol, a fatty acid, a cholesterol, an albumin or a fragment thereof, an albumin-binding substance, a polymer having

repeating units of a particular amino acid sequence, an antibody, an antibody fragment, an FcRn binding substance, an *in-vivo* connective tissue or a derivative thereof, a nucleotide, a fibronectin, a transferrin, an elastin-like polypeptide(ELP), an XTEN polypeptide, a carboxy-terminal peptide (CTP), a structure inducing probe (SIP), a saccharide and a high molecular weight polymer.

[16] In another specific example, the FcRn binding substance is characterized in that it includes an immunoglobulin Fc region.

[17] In another specific example, the physiologically active protein or peptide and the carrier are characterized by being bound via a linker interposed therebetween.

[18] In another specific example, the linker is characterized in that it is a non-peptidyl linker.

[19] In another specific example, the non-peptidyl linker is characterized in that it is selected from the group consisting of a polyethylene glycol, a polypropylene glycol, an ethylene glycol-propylene glycol copolymer, a polyoxyethylated polyol, a polyvinyl alcohol, a polysaccharide, a dextran, a polyvinyl ethyl ether, a biodegradable polymer, a lipid polymer, a chitin, a hyaluronic acid and a combination thereof.

[20] In another specific example, it is characterized in that the physiologically active protein or peptide is bound to an immunoglobulin Fc region via a non-peptidyl polymer which is selected from the group consisting of a polyethylene glycol, a polypropylene glycol, an ethylene glycol-propylene glycol copolymer, a poly-

oxyethylated polyol, a polyvinyl alcohol, a polysaccharide, a dextran, a polyvinyl ethyl ether, a biodegradable polymer, a lipid polymer, a chitin, a hyaluronic acid and a combination thereof.

[21] In another specific example, the physiologically active protein or peptide is characterized in that it is selected from the group consisting of an anti-obesity peptide, an insulintropic peptide or an analog thereof, a leptin, an insulin, an insulin analog, a glucagon, a human growth hormone, a growth hormone releasing hormone, a growth hormone releasing peptide, an interferon, an interferon receptor, a colony stimulating factor, a glucagon-like peptide such as GLP-1, a GLP-1/glucagon dual agonist, a gastric inhibitory polypeptide (GIP), a G-protein-coupled receptor, an interleukin, an interleukin receptor, an enzyme, an interleukin binding protein, a cytokine binding protein, a macrophage activating factor, a macrophage peptide, a B cell factor, a T cell factor, a protein A, an allergy inhibitory factor, a cell necrosis glycoprotein, an immunotoxin, a lymphotoxin, a tumor necrosis factor, a tumor inhibitory factor, a metastasis growth factor, an alpha-1 antitrypsin, an albumin, an α -lactalbumin, an apolipoprotein-E, an erythropoiesis factor, a highly glycosylated erythropoiesis factor, an angiopoietin, a hemoglobin, a thrombin, a thrombin receptor activating peptide, a thrombomodulin, blood factors VII, VIIa, VIII, IX and XIII, a plasminogen activating factor, a fibrin-binding peptide, an urokinase, a streptokinase, a hirudine, a protein C, C-reactive protein, a renin inhibitor, a collagenase inhibitor, a superoxide dismutase, a platelet-derived growth factor, an epithelial cell growth factor, an epidermal growth factor, an angiostatin, an angiotensin, a bone growth factor, a bone stimulating protein, a calcitonin, an atriopeptin, a cartilage inducing factor, an elcatonin, a connective tissue activating factor, a tissue factor pathway inhibitor, a follicle stimulating hormone, a luteinizing hormone, a luteinizing hormone releasing hormone, a nerve growth factor, a parathyroid hormone, a relaxin, a secretin, a somatomedin, an insulin-like growth factor, an adrenocortical hormone, a glucagon, a cholecystokinin, a pancreatic polypeptide, a gastrin-releasing peptide, a corticotropin releasing factor, a thyroid stimulating hormone, an autotaxin, a lactoferrin, a myostatin, a receptor, a receptor antagonist, a cell surface antigen, a virus-derived vaccine antigen, a monoclonal antibody, a polyclonal antibody, and an antibody fragment.

[22] In another specific example, the physiologically active protein or peptide is characterized in that it is selected from the group consisting of an exendin-4,

an exendin-4 derivative, a GLP-1 agonist, an insulin and a GLP-1/glucagon dual agonist.

[23] In another specific example, the exendin-4 derivative is characterized in that it is an exendin-4 derivative in which the charge on the N-terminal of exendin-4 is modified, which is selected from the group consisting of an exendin-4

derivative in which N-terminal amine group of exendin-4 is deleted, an exendin-4 derivative in which N-terminal amine group of exendin-4 is substituted with hydroxyl group, an exendin-4 derivative in which N-terminal amine group of exendin-4 is substituted with carboxyl group, an exendin-4 derivative in which N-terminal amine group of exendin-4 is modified with dimethyl group, and an exendin-4 derivative in which alpha carbon of N-terminal histidine residue of exendin-4 is deleted.

[24] In another specific example, the above-described internal residue is characterized in that it is a lysine residue at position 12 or 27 of the exendin-4 derivative in which N-terminal charge of exendin-4 is modified.

[25] In another specific example, the above-described internal residue is characterized in that it is a lysine residue at position 27 of the exendin-4 derivative in which N-terminal charge of exendin-4 is modified.

[26] In another specific example, the exendin-4 derivative in which the charge on the N-terminal of the exendin-4 is changed is characterized in that it is an exendin-4 derivative in which alpha carbon of N-terminal histidine residue of exendin-4 is deleted.

[27] Also disclosed herein is a composition, comprising a conjugate of a physiologically active protein or peptide in which a carrier is bound to the non-terminal, internal residue of a physiologically active protein or peptide, via a non-peptidyl linker, wherein the conjugate exhibits decreased immunogenicity as compared to that of the physiologically active protein or peptide to which the carrier is not bound.

[28] In one specific example, the above-described conjugate is characterized in that it has decreased immunogenicity, which is a side effect of a long-acting preparation.

[29] In another specific example, the non-peptidyl linker is characterized in that it is a polyethylene glycol.

[30] Also disclosed herein is a method for preparing the conjugate of the physiologically active protein or peptide, in which the carrier is bound to the non-terminal, internal residue of the physiologically active protein or peptide.

[31]

【Advantageous Effects of Invention】

[32] The physiologically active protein or peptide conjugate of the present invention can significantly decrease immunogenicity in the human body and thus reduce antibody production rate against proteins or peptides. Therefore, the present conjugate has advantages in that the phenomenon of reduced clinical effects of the physiologically active protein or peptide is low, and it can be effectively used in the development of

Claims

1. A method for decreasing immunogenicity of a physiologically active protein or peptide as compared to that of a physiologically active protein or peptide to which a carrier is not bound, which comprises inhibiting the mechanism in which the desired protein or peptide acts as an antigen by binding a carrier to a non-terminal, internal residue of the physiologically active protein or peptide, wherein the physiologically active protein or peptide is selected from the group consisting of an exendin-4, an exendin-4 derivative, a GLP-1, a GLP-1 agonist, an insulin and a GLP-1/glucagon dual agonist; and wherein the carrier is a polyethylene glycol, or an immunoglobulin Fc region.
2. The method according to claim 1, further comprising inhibiting T cell proliferation.
3. The method according to claim 1, further comprising inhibiting secretion of interleukin-2.
4. The method according to claim 1, wherein the physiologically active protein or peptide and the carrier are bound via a non-peptidyl linker interposed therebetween.
5. The method according to claim 4, wherein the non-peptidyl linker is selected from the group consisting of a polyethylene glycol, a polypropylene glycol, an ethylene glycol-propylene glycol copolymer, a polyoxyethylated polyol, a polyvinyl alcohol, a polysaccharide, a dextran, a polyvinyl ethyl ether, a biodegradable polymer, a lipid polymer, a chitin, a hyaluronic acid and a combination thereof.
6. The method according to claim 4, wherein the physiologically active protein or peptide is bound to an immunoglobulin Fc region via a non-peptidyl polymer which is selected from the group consisting of a polyethylene glycol, a polypropylene glycol, an ethylene glycol-propylene glycol copolymer, a polyoxyethylated polyol, a polyvinyl alcohol, a polysaccharide, a dextran, a polyvinyl ethyl ether, a biodegradable polymer, a lipid polymer, a chitin, a hyaluronic acid and a combination thereof.
7. The method according to claim 1, wherein the physiologically active protein or peptide is an exendin-4, or an exendin-4 derivative.

8. The method according to claim 7, wherein the exendin-4 derivative is an exendin-4 derivative in which the charge on the N-terminal of exendin-4 is modified, which is selected from the group consisting of an exendin-4 derivative in which N-terminal amine group of exendin-4 is deleted; an exendin-4 derivative in which N-terminal amine group of exendin-4 is substituted with hydroxyl group; an exendin-4 derivative in which N-terminal amine group of exendin-4 is substituted with carboxyl group; an exendin-4 derivative in which N-terminal amine group of exendin-4 is modified with dimethyl group; and an exendin-4 derivative in which alpha carbon of N-terminal histidine residue of exendin-4 is deleted.
9. The method according to claim 7 or 8, wherein the internal residue is a lysine residue at position 12 or 27 of the exendin-4 derivative in which N-terminal charge of exendin-4 is modified.
10. The method according to claim 9, wherein the internal residue is a lysine residue at position 27 of the exendin-4 derivative in which N-terminal charge of exendin-4 is modified.
11. The method according to claim 9, wherein the exendin-4 derivative in which the charge on the N-terminal of the exendin-4 is modified is an exendin-4 derivative in which alpha carbon of N-terminal histidine residue of exendin-4 is deleted.
12. A composition, comprising a conjugate of a physiologically active protein or peptide in which a carrier is bound to the non-terminal, internal residue of a physiologically active protein or peptide, via a non-peptidyl linker, wherein the conjugate inhibits the mechanism in which the desired protein or peptide acts as an antigen and exhibits decreased immunogenicity as compared to that of the physiologically active protein or peptide to which the carrier is not bound, wherein the physiologically active protein or peptide is selected from the group consisting of an exendin-4, an exendin-4 derivative, a GLP-1, a GLP-1 agonist, an insulin and a GLP-1/glucagon dual agonist; and wherein the carrier is a polyethylene glycol, or an immunoglobulin Fc region.
13. The composition according to claim 12, wherein the conjugate has decreased immunogenicity, which is a side effect of a long-acting preparation.
14. The composition according to claim 12, wherein the non-peptidyl linker is a polyethylene glycol.