

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; and inhibiting T cell proliferation and secretion of interleukin-2, wherein the physiologically active protein or peptide is selected from the group consisting of an exendin-4, an exendin-4 derivative, a glucagon-like peptide-1(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 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. In yet another aspect, the present invention provides a composition for decreasing immunogenicity of a physiologically active protein or peptide, comprising a carrier capable of binding to the non-terminal, internal residue of the physiologically active protein or peptide, and forming a conjugate with the physiologically active protein or peptide, wherein the conjugate 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 glucagon-like peptide-1(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 yet another aspect, the present invention provides use of a carrier in decreasing immunogenicity of a physiologically active protein or peptide as compared to that of a 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 glucagon-like peptide-1(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 there between.

[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-

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; and
inhibiting T cell proliferation and secretion of interleukin-2,
wherein the physiologically active protein or peptide is selected from the group consisting of an exendin-4, an exendin-4 derivative, a glucagon-like peptide-1(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, wherein the physiologically active protein or peptide and the carrier are bound via a non-peptidyl linker interposed therebetween.
3. The method according to claim 2, 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.
4. The method according to claim 2, 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.
5. The method according to claim 1, wherein the physiologically active protein or peptide is an exendin-4, or an exendin-4 derivative.
6. The method according to claim 5, 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.

7. The method according to claim 5 or 6, 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.

8. The method according to claim 7, 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.

9. The method according to claim 7, 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.

10. 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.

11. The composition according to claim 10, wherein the conjugate has decreased immunogenicity, which is a side effect of a long-acting preparation.

12. The composition according to claim 10, wherein the non-peptidyl linker is a polyethylene glycol.

13. A composition for decreasing immunogenicity of a physiologically active protein or

peptide, comprising a carrier capable of binding to the non-terminal, internal residue of the physiologically active protein or peptide, and forming a conjugate with the physiologically active protein or peptide,

wherein the conjugate 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 glucagon-like peptide-1(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.

14. Use of a carrier in decreasing immunogenicity of a physiologically active protein or peptide as compared to that of a 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 glucagon-like peptide-1(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.